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ORIGINAL RESEARCH

Genetic Overlap Between Obstructive Sleep Apnea and Ischemic Stroke: A Large-Scale Genome-Wide Cross-Trait Analysis

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Background: To further understand the complex relationship between Obstructive Sleep Apnea (OSA) and ischemic stroke, this study explores the role of genetic factors in the comorbidity of these two conditions.

Methods: Based on large-scale available Genome-Wide Association Studies (GWAS) for OSA and ischemic stroke, we conducted a multi-level cross-trait analysis. First, we utilized Linkage Disequilibrium Score Regression (LDSC) to analyze the genetic correlation between the two diseases. Subsequently, we performed cross-trait analysis to identify pleiotropic Single Nucleotide Polymorphisms (SNPs) associated with both OSA and ischemic stroke. On this basis, we applied annotation and Multi-marker Analysis of GenoMic Annotation (MAGMA) analysis to examine results at the gene level. Finally, we conducted Transcriptome-Wide Association Studies (TWAS) to analyze gene expressions significantly related to both traits.

Results: The LDSC analysis revealed a significant positive genetic correlation between OSA and ischemic stroke. Cross-trait analysis identified a total of 90 pleiotropic SNPs, with rs78581380 being the most significant. Combining Functional Mapping and Annotation (FUMA) annotation and MAGMA analysis, we identified 83 genes in total. TWAS analysis discovered 23 gene expressions that were significantly associated with both OSA and ischemic stroke traits.

Conclusion: This study elucidates the shared genetic architecture between OSA and ischemic stroke, emphasizing the crucial role of genetic factors in the comorbidity of these two conditions.

Keywords: obstructive sleep apnea, ischemic stroke, GWAS, genetic overlap

Introduction

Stroke is an acute condition caused by the sudden interruption of blood supply to the brain, leading to the death of brain cells due to lack of oxygen, potentially resulting in permanent disability, speech impairment, or even life-threatening consequences. As of 2019, there were approximately 12.2 million new cases of stroke worldwide, which has placed a significant burden on both nations and families.[1] Due to its severe impact on quality of life, identifying the risk factors of stroke is crucial for early diagnosis and treatment. Obstructive Sleep Apnea (OSA) is a sleep disorder where the upper airway is partially or completely blocked during sleep, causing reduced or stopped airflow.[2] This can lead to problems with attention and memory. In recent years, OSA has garnered increasing attention due to its significant health risks and its close association with various other diseases.[3–5]

Previous studies have demonstrated that patients with OSA are at an increased risk of stroke, even after adjusting for confounding factors such as hypertension and diabetes.[6] This heightened risk may be attributed to intermittent hypoxemia caused by OSA, which promotes oxidative stress and systemic inflammation, ultimately resulting in endothelial

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dysfunction.[7,8] Furthermore, excessive activation of the sympathetic nervous system induced by OSA leads to recurrent elevations in blood pressure, thereby increasing the risk of cardiovascular diseases and stroke.[9] Sahlin et, in a 10-year follow-up study, reported that stroke patients with comorbid OSA exhibited a significantly higher risk of mortality.[10] Meanwhile, Blissitt et al found that stroke-related neurological deficits, such as reduced pharyngeal tone or impaired ventilatory drive, may contribute to the development of OSA.[11] Post-stroke OSA is associated with poorer neurological recovery and increased long-term mortality.[12] Despite numerous studies confirming an association between OSA and stroke, the evidence for a causal relationship between OSA and cardiovascular diseases remains inconclusive. Fava et al suggested that OSA might merely reflect traditional cardiovascular risk factors—such as obesity, diabetes, and hyperlipidemia—rather than acting as an independent risk factor.[13] Additionally, confounding variables may influence these findings; for example, the association between OSA and coronary heart disease diminishes after adjusting for body mass index.[14] While multiple studies indicate that continuous positive airway pressure (CPAP) therapy can improve blood pressure control in OSA patients and potentially reduce cardiovascular risks, there remains a lack of long-term, large-scale randomized controlled trials to definitively determine whether CPAP significantly lowers the incidence of cardiovascular events.[15,16]

Genetic and environmental factors are critical contributors to the development of diseases.[17,18] For instance, genetic variations that regulate upper airway muscle tone, blood pressure, and blood coagulation mechanisms can increase the risk of OSA.[19] Moreover, genetic factors are estimated to account for 30–40% of stroke risk.[20] Traditional genetic studies often focus on the impact of genetic variations on a single specific disease or trait. However, research on genetic overlap has revealed shared genetic links and mechanisms between different diseases. By integrating genetic data across multiple diseases, these studies can identify complex, multilayered genetic factors, thereby uncovering deeper pathological mechanisms that complement the limitations of clinical research.[21]

Current research lacks a comprehensive understanding of the genetic overlap and underlying mechanisms linking OSA and stroke. With the advancement of genome-wide association studies (GWAS) related to OSA[22] and stroke,[23] this paper aims to investigate the shared genetic architecture between these two conditions. We first employed Linkage Disequilibrium Score Regression (LDSC) to analyze the genetic correlation between OSA and stroke. Following this, we utilized cross-trait analysis to explore the genetic overlap at the SNP, genetic, and mRNA levels. This approach seeks to provide a more comprehensive understanding of the genetic interplay between OSA and stroke, which could offer new insights into their underlying pathological mechanisms.

Method

We obtained summary GWAS data for OSA, which included 38,998 cases and 336,659 controls, from the FinnGen consortium R9 (https://www.finngen.fi/en/access_results). The ischemic stroke data were sourced from a recent large-scale GWAS, comprising 62,100 cases and 1,234,808 controls, available from the GWAS Catalog (https://www.ebi.ac.uk/gwas/publications/36180795). The criteria for selecting GWAS data were as follows: studies with as large a sample size as possible, accessible data from European-ancestry cohorts, and, where feasible, databases for the two traits from different sources. The data underwent a rigorous cleaning process, including standardization of the major effect alleles, removal of duplicate SNPs, and supplementation of essential genetic information, such as rsid numbers and chromosome positions, where missing. The cleaned GWAS data were then aligned with the European 1000 Genomes Project to ensure consistency and accuracy across shared regions (Figure 1).

Linkage Disequilibrium Score Regression (LDSC) is a widely used genomic analysis method that estimates genetic contributions to phenotypic variation by leveraging linkage disequilibrium (LD) information from GWAS. This approach distinguishes genetic effects from confounding environmental influences by regressing GWAS summary statistics on LD scores across the genome.[24,25] LDSC provides estimates of heritability (h^2) for individual traits and evaluates genetic correlation (rg) between traits, offering insights into shared genetic architectures. By performing regression on LD scores across different regions of the genome, LDSC can estimate the $h^2$of a particular phenotype and assess the distribution of genetic effects.[26] This analysis enabled us to quantify the extent to which common genetic variants contribute to both conditions, providing valuable evidence for their shared genetic basis.

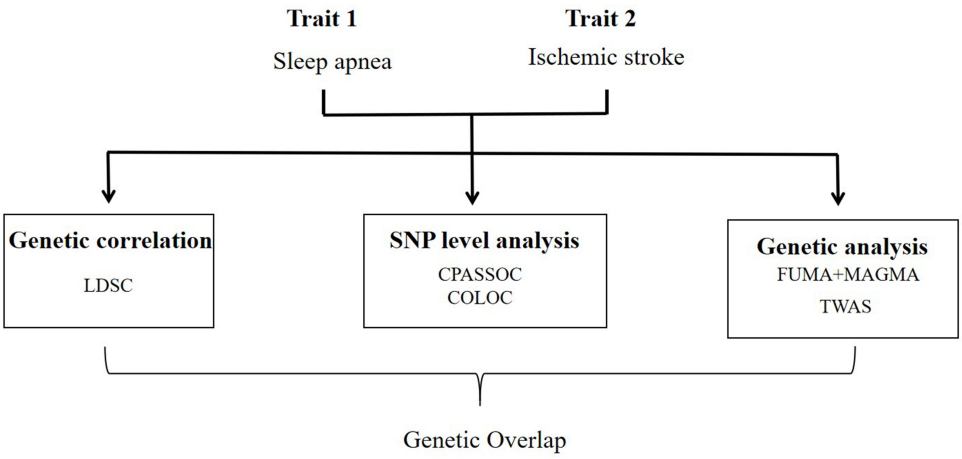

Figure 1 Overview of research of shared genetic architecture between OSA and ischemic stroke.

Cross-Phenotype Association (CPASSOC) analysis is a crucial method for investigating the shared genetic foundations across different phenotypes. Unlike traditional single-phenotype association analyses, CPASSOC allows for the simultaneous evaluation of genetic associations across multiple phenotypes, thereby identifying genetic variants that may be common among them.[26] This method, based on meta-analysis, integrates GWAS results from various phenotypes to uncover shared genetic variants. By utilizing two statistical measures, SHom (homogeneity) and SHet (heterogeneity), CPASSOC effectively detects genetic variants associated with multiple phenotypes, even when there is heterogeneity in effect sizes among the phenotypes.

Further investigation is needed to determine whether the two traits are influenced by the same genetic variants or by distinct but closely linked ones. We performed colocalization analysis (COLOC) using a Bayesian algorithm. In the COLOC analysis, five hypotheses are considered: Hypothesis (H) 0, where the genetic variation in the region is not associated with either trait; H1, where the genetic variation is associated with trait 1 but not with trait 2; H2, where the genetic variation is associated with trait 2 but not with trait 1; H3, where the genetic variation is associated with both traits but through different genetic variants; and H4, where the same genetic variant is associated with both traits.[27]

Additionally, gene-level analysis can aggregate the effects of multiple SNPs within a gene, which may individually have minimal or insignificant effects, thereby increasing the power to detect meaningful genetic associations. We subsequently analyzed pleiotropic genes associated with both OSA and neck pain. Gene- and trait-based association analyses were conducted using Multi-marker Analysis of GenoMic Annotation (MAGMA), employing principal component regression models in combination with the 1000 Genomes European reference sample to enhance detection power.[28] It employed principal component regression models alongside the 1000 Genomes European reference panel to enhance the detection power of genetic associations. This approach allowed us to integrate LD patterns, improving the accuracy of gene-level association estimates. By leveraging MAGMA, we ensured a more comprehensive analysis of the shared genetic mechanisms underlying OSA and stroke. We then utilized Functional Mapping and Annotation (FUMA) to identify SNPs located in close proximity within genomic regions and assigned them to their corresponding genes. Finally, we identified the overlapping genes from both methods and performed corrections to establish the final results.

The intricate interplay between genetic variations and gene expression regulation is fundamental to the manifestation of complex traits in populations. Understanding this relationship is crucial for unraveling the genetic architecture underlying these traits and for developing targeted therapeutic interventions. A transcriptome-wide association study (TWAS) first constructs transcript-level prediction models using genetic variations near genes, which are then employed to estimate gene expression levels in individuals. Finally, TWAS performs thorough association analyses between the predicted gene expression levels and phenotypic traits to identify significant correlations.We performed TWAS using tissue expression weights from the GTEx database (version 8) through the Functional Summary-based Imputation (FUSION) platform (http://gusevlab.org/projects/fusion/). Given that our analysis focuses on neurological disorders,

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we included brain tissues from different anatomical locations as reference templates. The GTEx database (version 8) includes gene expression data from 13 brain tissues, representing diverse functional and structural regions of the brain. These tissues include the amygdala, anterior cingulate cortex (BA24), caudate (basal ganglia), cerebellar hemisphere, cerebellum, cortex, frontal cortex (BA9), hippocampus, hypothalamus, nucleus accumbens (basal ganglia), putamen (basal ganglia), spinal cord (cervical c-1), and substantia nigra. This comprehensive dataset provides valuable insights into gene expression patterns across key neurological regions.

Mendelian Randomization (MR) is an analytical method that uses genetic variants as instrumental variables (IVs) to assess the causal relationship between an exposure and an outcome. This method relies on three key assumptions: (1) the genetic variants are strongly associated with the exposure; (2) the genetic variants are independent of confounding factors; and (3) the genetic variants influence the outcome only through the exposure. In this study, the IVs were selected based on a significance threshold of $P<5\times10^{-8}$. To minimize LD, we applied the following parameters: Kb=10,000 and r2=0.0001. Genetic variants with an F-statistic greater than 10 were considered strong instruments. For sensitivity analyses, we performed tests for horizontal pleiotropy and heterogeneity to ensure the robustness of our causal inference. These analyses help to evaluate potential violations of MR assumptions and increase the reliability of the results.

Result

General Genetic Correlation

The h^2 of ischemic stroke was 0.0062, and that of OSA was 0.0437. A significant positive genetic correlation between ischemic stroke and OSA had been identified, with LDSC analysis revealing a rg of 0.209 and a p-value of 5.116E-08.

SNP Level Analysis

The CPASSOC analysis identified a total of 90 pleiotropic SNPs associated with both OSA and ischemic stroke, with individual trait p-values satisfying P<1E-03 and a meta-analysis p-value (Pmeta)<5E-08 (Supplementary Table 01). The most significant SNP identified, rs78581380, was located at chromosome position 6:1365073, with P_{OSA}=9.167E-04, $P_{ischemic\ stroke}$=1.30E-13, and P_{meta}=4.91E-16. Additionally, we discovered six novel pleiotropic SNPs with P-values between 5E-08 and 1E-03 for single trait of OSA and ischemic stroke. Among these, the most significant SNP was rs891512, located at CHR:BP=7:150708089, with P_{OSA}=2.89E-06, $P_{ischemic\ stroke}$=1.61E-06, and Pmeta=1.43E-08. Notably, this SNP, along with 4 others (rs2256314, rs1808593, rs743507, rs743506), was located within the *NOS3* gene (Table 1). Nitric Oxide Synthase 3 encodes endothelial nitric oxide synthase (eNOS), which synthesizes nitric oxide in endothelial cells, regulating vascular tone and blood pressure, and is crucial for cardiovascular health.[29] The COLOC analysis identified a genomic region on chromosome 7 (CHR: BP = 7:150,688,083–150721586) with a posterior probability of 0.80, indicating a strong shared genetic association between OSA and stroke.

Table 1 The Novel Pleiotropic SNPs Between OSA and Ischemic Stroke Identified from Cross-Trait Meta-Analysis

SNP	CHR	BP	A1	A2	BETA	SE	P	BETA	SE	P	Test.shet	
					Trait1:Ischemic stroke			Trait2:OSA			CPASSOC	
rs891512	7	150708089	A	G	0.045	0.009	1.61E-06	0.050	0.011	2.89E-06	33.72	1.43E-08
rs2256314	7	150708850	G	A	0.042	0.009	2.78E-06	0.052	0.010	5.84E-07	33.54	1.58E-08
rs1808593	7	150708302	G	T	0.041	0.009	3.97E-06	0.052	0.010	4.20E-07	32.98	2.12E-08
rs743507	7	150707488	C	T	0.041	0.009	4.61E-06	0.052	0.010	4.33E-07	32.52	2.71E-08
rs743506	7	150706915	G	A	0.04	0.009	7.03E-06	0.052	0.010	5.19E-07	31.63	4.34E-08
rs7746700	6	1323447	G	A	0.033	0.007	7.06E-06	−0.035	0.008	1.02E-05	31.37	4.99E-08

Genetic Level Analysis

By utilizing FUMA annotation alongside the MAGMA method (Supplementary Table 02 and 03), we identified 93 pleiotropic genes (Supplementary Table 04). After applying Bonferroni correction, where the threshold was adjusted to $P < 0.05/93 = 5.81E-04$, 83 genes remained significant (Supplementary Table 05). The most significant gene associated with both traits is *FOXF2*, located at CHR:BP = 6:1,340,069–1445832, with a P_{MAGMA} of 3.06E-13. *FOXF2*, also known as Forkhead Box F2, encodes a transcription factor that is critical for the development of the nervous, digestive, and cardiovascular systems during embryogenesis.[30] Dysfunction in the *FOXF2* gene may result in cerebral hemorrhage, making it a susceptible genetic factor for ischemic stroke.[31] The second gene significantly associated with both traits is *FOXQ1*, another member of the forkhead box transcription factor family. It is located at CHR:BP = 6:1,262,675~1364992, with a P_{MAGMA} of 5.20E-13. The protein it encodes plays a crucial role in cell differentiation, proliferation, and development.[32]

TWAS

In the TWAS study results (Figure 2), we found that a total of 23 gene expressions were significantly associated with both OSA and ischemic stroke traits (Table 2). Among them, the gene GAPVD1 was also identified as a pleiotropic gene for

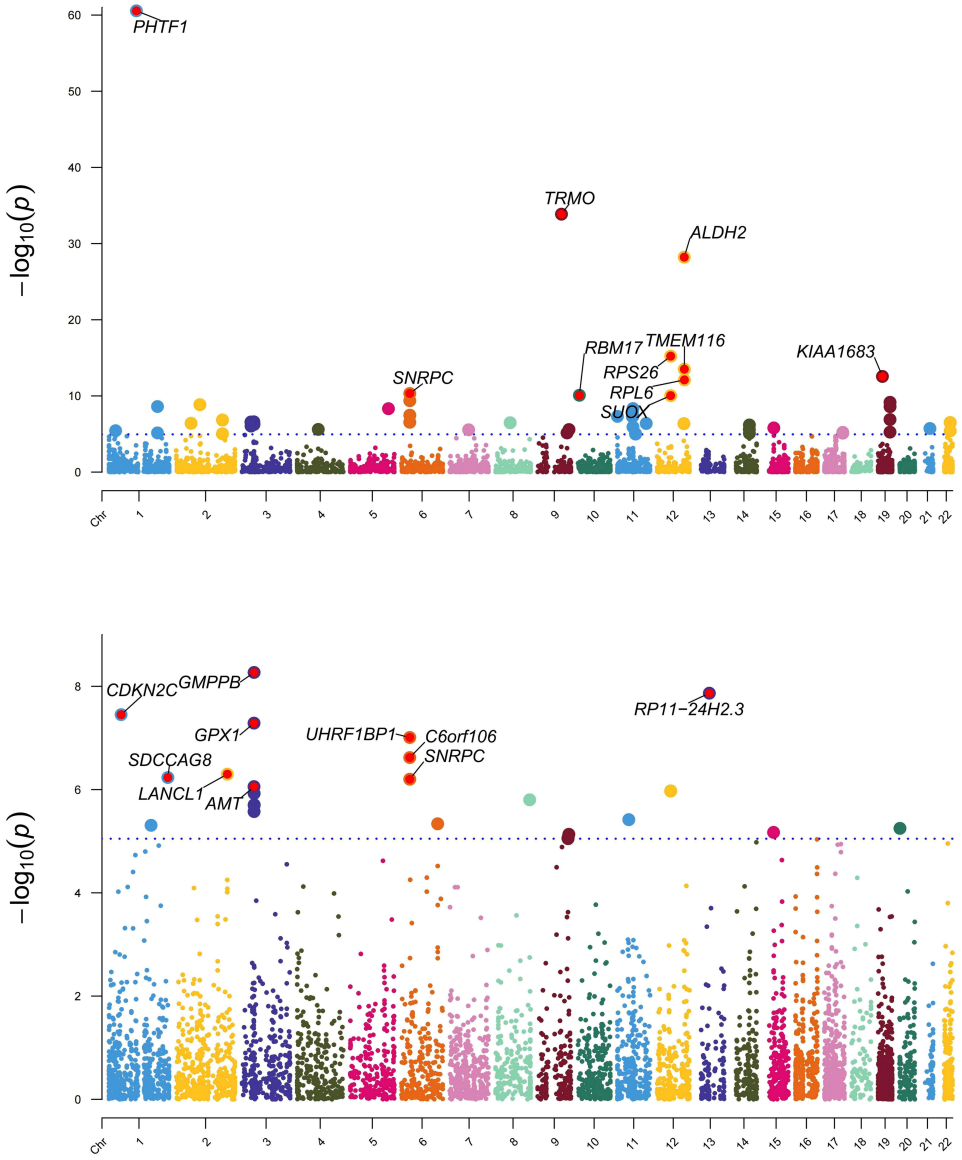

Figure 2 The Manhattan plot of the GWAS of ischemic stroke and the Manhattan plot of the GWAS of OSA.

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Table 2 The TWAS Results of the Genes Significantly Expressed in Both OSA and Ischemic Stroke Across Different Tissues

Tissue	ID	TWAS.Z.1	TWAS.P.1	TWAS.fdr.P.1	TWAS.Z.2	TWAS.P.2	TWAS.fdr.P.2
		1.Ischemic stroke			2.OSA		
GTExv8.EUR.Brain_Caudate_basal_ganglia	*CSDC2*	−5.15	2.66E-07	4.60E-05	4.07	4.79E-05	1.26E-02
	EFCAB13	−3.78	1.58E-04	6.96E-03	−4.01	6.08E-05	1.40E-02
	EP300-AS1	−3.19	1.40E-03	3.51E-02	4.31	1.67E-05	8.36E-03
	EPHB2	−3.08	2.09E-03	4.66E-02	3.58	0.000344	3.83E-02
	NDUFA6	−3.29	1.00E-03	2.71E-02	4.37	1.24E-05	8.36E-03
	NPEPPS	4.93	8.15E-07	1.08E-04	4.34	1.41E-05	8.36E-03
	POLR3H	−5.28	1.30E-07	2.72E-05	4.21	2.58E-05	1.10E-02
	RALGAPA1	−3.60	3.16E-04	1.17E-02	3.70	0.000216	3.09E-02
	TRMO	12.27	1.38E-34	3.46E-31	3.62	3.00E-04	3.55E-02
	XRCC6	5.16	2.53E-07	4.54E-05	−4.14	3.44E-05	1.10E-02
GTExv8.EUR.Brain_Amygdala	*CSDC2*	−5.54	3.03E-08	9.82E-06	3.89	0.000102	3.49E-02
	NDUFA6	−3.29	1.00E-03	2.64E-02	4.37	1.24E-05	1.60E-02
	NPEPPS	7.28	3.26E-13	2.11E-10	3.80	0.000143	3.70E-02
	POLR3H	−5.27	1.36E-07	3.53E-05	4.23	2.33E-05	2.01E-02
	TRMO	12.27	1.38E-34	3.58E-31	3.62	3.00E-04	4.85E-02
GTExv8.EUR.	*CSDC2*	−5.49	3.99E-08	1.37E-05	3.89	9.89E-05	1.65E-02
Brain_Anterior_cingulate_cortex_BA24	*EFCAB13*	−4.67	2.98E-06	3.66E-04	−4.44	9.12E-06	9.42E-03
	MEI1	−5.28	1.32E-07	3.14E-05	4.05	5.04E-05	1.44E-02
	POLR3H	−5.03	4.88E-07	7.98E-05	4.16	3.16E-05	1.44E-02
	RP11-3B7.1	−3.10	1.92E-03	4.17E-02	−4.75	2.02E-06	6.92E-03
	TRMO	12.27	1.38E-34	4.74E-31	3.62	3.00E-04	3.21E-02
GTExv8.EUR.Brain_Putamen_basal_ganglia	*EFCAB13*	−4.67	2.98E-06	3.03E-04	−4.44	9.12E-06	7.80E-03
	MEI1	−4.94	7.90E-07	1.09E-04	4.15	3.34E-05	8.96E-03
	POLR3H	−5.46	4.86E-08	1.15E-05	4.12	3.75E-05	8.96E-03
	RALGAPA1	−3.66	2.48E-04	9.53E-03	3.62	0.000291	3.75E-02
	TBKBP1	3.04	2.38E-03	4.77E-02	4.08	4.59E-05	1.03E-02
	TRMO	12.27	1.38E-34	5.89E-31	3.62	3.00E-04	3.75E-02
GTExv8.EUR.	*CSDC2*	−5.09	3.63E-07	7.16E-05	4.01	6.06E-05	2.43E-02
Brain_Nucleus_accumbens_basal_ganglia	*NDUFA6*	−3.34	8.23E-04	2.45E-02	4.15	3.32E-05	2.43E-02
GTExv8.EUR.Brain_Hypothalamus	*CSDC2*	−5.21	1.84E-07	3.41E-05	4.23	2.30E-05	1.35E-02
	EFCAB13	−3.58	3.48E-04	1.03E-02	−4.37	1.26E-05	1.11E-02
	GPC2	3.15	1.65E-03	3.42E-02	−3.62	0.000299	3.66E-02
	MEI1	−5.28	1.32E-07	2.74E-05	4.05	5.04E-05	1.77E-02
	MRPL45P2	−3.09	2.01E-03	3.96E-02	−4.53	5.83E-06	1.02E-02
	POLR3H	−4.98	6.44E-07	9.46E-05	4.07	4.61E-05	1.77E-02
	RALGAPA1	−3.59	3.29E-04	1.00E-02	3.71	0.00021	3.52E-02
	RP11-290H9.5	3.57	3.60E-04	1.05E-02	4.44	9.16E-06	1.07E-02
	TRMO	12.27	1.38E-34	4.86E-31	3.62	3.00E-04	3.66E-02
	EFCAB13	−4.81	1.51E-06	1.98E-04	−4.79	1.68E-06	2.96E-03
	NPEPPS	5.38	7.54E-08	2.05E-05	4.94	7.97E-07	2.81E-03
	POLR3H	−4.91	9.09E-07	1.34E-04	4.11	4.03E-05	2.37E-02
GTExv8.EUR.Brain_Cortex	*ATP6V0A2*	−3.49	4.89E-04	1.72E-02	−4.06	4.82E-05	1.35E-02
	EFCAB13	−3.58	3.46E-04	1.31E-02	−4.42	1.00E-05	4.64E-03
	MEI1	−5.13	2.87E-07	5.94E-05	4.19	2.79E-05	9.71E-03
	MRPL45P2	−3.04	2.38E-03	4.87E-02	−4.08	4.59E-05	1.35E-02
	NDUFA6	−3.37	7.64E-04	2.27E-02	3.81	0.000142	2.40E-02
	NPEPPS	4.53	5.81E-06	6.49E-04	5.45	5.16E-08	2.87E-04
	RALGAPA1	−3.60	3.19E-04	1.26E-02	3.71	0.000209	3.23E-02
	TBKBP1	3.28	1.02E-03	2.71E-02	4.66	3.16E-06	2.77E-03
	TRMO	12.27	1.38E-34	7.71E-31	3.62	3.00E-04	4.08E-02

(*Continued*)

Table 2 (Continued).

Tissue	ID	TWAS.Z.1	TWAS.P.1	TWAS.fdr.P.1	TWAS.Z.2	TWAS.P.2	TWAS.fdr.P.2
		1.Ischemic stroke			2.OSA		
GTExv8.EUR.Brain_Cerebellum	*ACO2*	−5.26	1.43E-07	3.47E-05	4.29	1.78E-05	6.13E-03
	EP300	−3.65	2.59E-04	1.14E-02	4.02	5.88E-05	1.65E-02
	GAPVD1	−3.90	9.61E-05	5.45E-03	−5.43	5.57E-08	2.01E-04
	POLR3H	−5.00	5.73E-07	1.04E-04	4.08	4.56E-05	1.37E-02
	RALGAPA1	−3.58	3.40E-04	1.46E-02	3.66	0.000255	4.02E-02
	RP1-257I20.14	−3.18	1.48E-03	3.83E-02	3.94	8.07E-05	1.94E-02
	TRMO	12.27	1.38E-34	3.02E-31	3.62	3.00E-04	4.28E-02
	ZC3H7B	−3.83	1.30E-04	6.73E-03	4.31	1.64E-05	6.13E-03
GTExv8.EUR.Brain_Cerebellar_Hemisphere	*GAPVD1*	−3.55	3.87E-04	1.32E-02	−5.34	9.48E-08	5.74E-04
	POLR3H	−4.76	1.89E-06	2.34E-04	3.97	7.27E-05	2.63E-02
	RALGAPA1	−3.60	3.24E-04	1.15E-02	3.72	0.000201	3.89E-02
	THCAT158	4.71	2.53E-06	3.07E-04	4.12	3.78E-05	2.08E-02
	TRMO	12.27	1.38E-34	2.68E-31	3.62	3.00E-04	3.89E-02
	XRCC6	4.97	6.64E-07	9.91E-05	−4.17	3.01E-05	2.00E-02

OSA and stroke in the previous gene-level analysis. These gene expressions were concentrated in the caudate basal ganglia, amygdala, anterior cingulate cortex, putamen basal ganglia, nucleus accumbens basal ganglia, hypothalamus, hippocampus, cortex, cerebellum, and cerebellar hemisphere. Among these, the genes with the most widespread expression across tissues were *POLR3H* and *TRMO*, which were found to be significantly associated with both OSA and ischemic stroke in eight different tissues. *POLR3H*, as a subunit of RNA polymerase III, plays an important role in the transcription of small non-coding RNAs, which are crucial for various cellular processes.[33] *TRMO* (tRNA Methyltransferase O) is an enzyme that modifies tRNA molecules through methylation, ensuring their proper functioning and stability during protein synthesis.[34] The genes with the second-most widespread tissue expression were *EFCAB13* and *RALGAPA1*. *EFCAB13* stands for EF-hand Calcium Binding Domain 13, playing a role in calcium signaling due to its EF-hand motifs, which are known to bind calcium ions.[34] *RALGAPA1* encodes RAL GTPase-Activating Protein Alpha 1, a component of the RalGAP complex, which functions as a GTPase-activating protein (GAP) for Ral GTPases, specifically RALA and RALB. By regulating the activity of these GTPases, *RALGAPA1* plays a critical role in maintaining cellular homeostasis and modulating cellular responses.[35]

MR

When stroke was analyzed as the exposure and OSA as the outcome, a total of 19 IVs were identified, all with F-statistics greater than 10. Using the Inverse Variance Weighted (IVW) method, the analysis yielded P=0.33, OR=1.06, and a 95% confidence interval (CI) of 0.94–1.19. The heterogeneity test showed P=0.71, indicating no significant heterogeneity (P>0.05). However, the horizontal pleiotropy test yielded P=0.02, suggesting potential pleiotropy. To address this, we applied a random-effects model, which produced results of P=0.38. In the reverse analysis, where OSA was considered the exposure and stroke the outcome, 16 IVs were identified. The IVW method produced results of P=0.39, OR=1.03, and a 95% confidence interval (CI) of 0.95–1.11. Both the heterogeneity test (P=0.08) and the horizontal pleiotropy test (P=0.47) indicated no evidence of significant heterogeneity or pleiotropy (P>0.05) (Figure 3).

Discussions

As far as we know, this is the first study to investigate the shared genetic architecture between OSA and stroke. First, we used two methods to discover a significant genetic correlation between OSA and stroke. Then, using cross-trait analysis, we identified pleiotropic SNPs and genes shared by both conditions. Finally, we employed the TWAS method to analyze

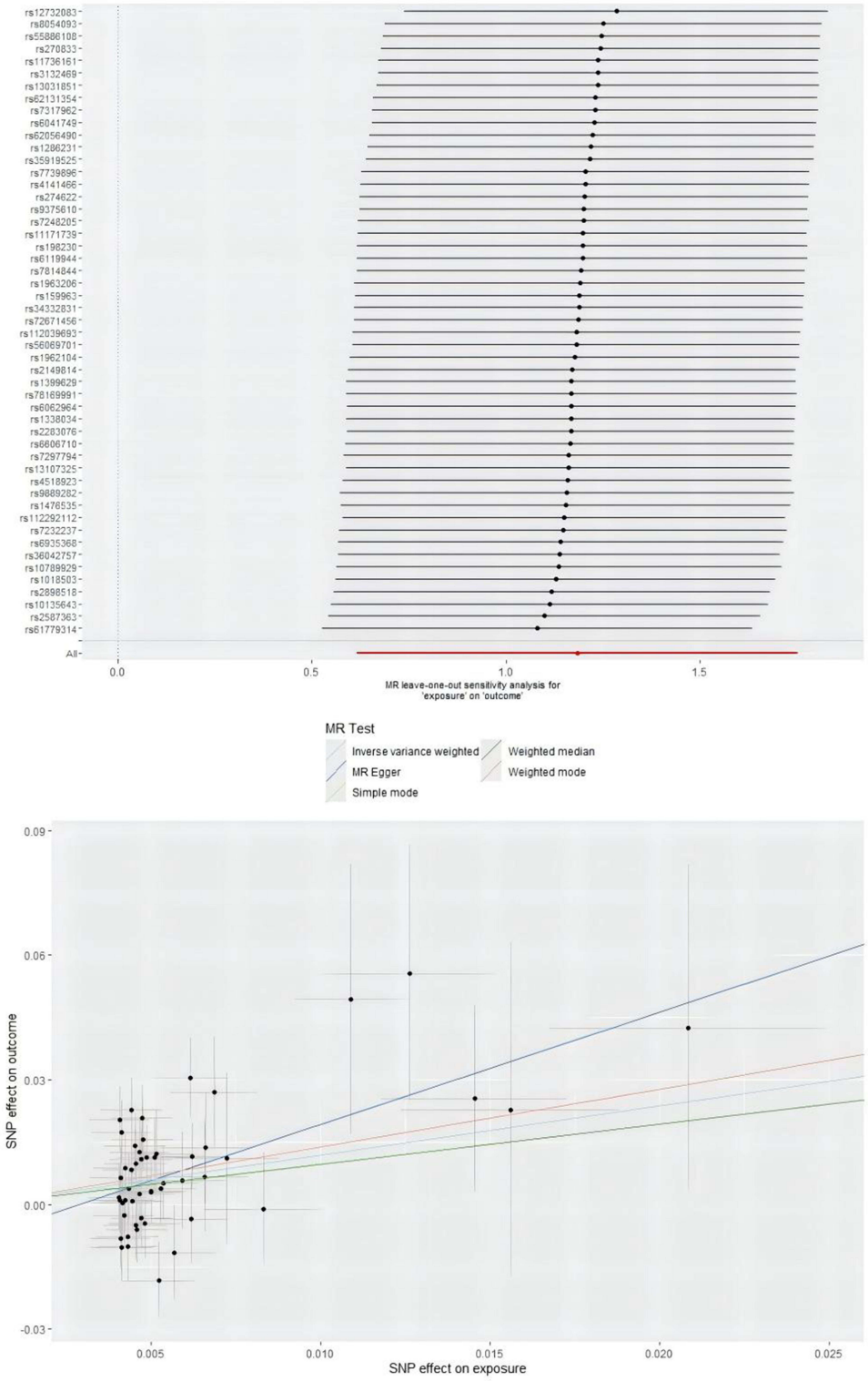

Figure 3 The scatter plot and the leave-one-out plot for the causal association between stroke and OSA.

genes that are significantly expressed in both traits. These results indicate that OSA and stroke share common pathological mechanisms at the genetic level, providing new insights into the shared etiology of these two diseases.

The results of the LDSC study found a significant positive correlation between OSA and ischemic stroke, consistent with previous clinical findings. This suggests that genetic factors play a role in the comorbidity. Furthermore, through

cross-trait analysis, we discovered pleiotropic genes associated with both OSA and ischemic stroke. In the colocalization study, we identified a locus related to *NOS3* and *ATG9B*, both of which are associated with OSA and ischemic stroke.

Nitric Oxide Synthase 3 (*NOS3*) is a gene that encodes endothelial nitric oxide synthase, an enzyme primarily expressed in vascular endothelial cells that catalyzes the production of nitric oxide (NO). Mutations in the *NOS3* gene may increase the risk of developing various cardiovascular diseases, including atherosclerosis, ischemic stroke, and hypertension.[36] In OSA, intermittent hypoxia and oxidative stress are known to impair endothelial NO production, exacerbating vascular inflammation and promoting atherosclerosis.[37] This vascular dysfunction is also a key factor in ischemic stroke. Therefore, the pleiotropic effects of NOS3 may link the pathophysiology of OSA and ischemic stroke through shared mechanisms of vascular dysfunction and inflammation.[38] In a community-based cohort study of children, a significant difference in the *NOS3* gene was identified between the non-OSA group and the OSA group.[39] At the same time, the expression of the *NOS3* gene is also influenced by inflammatory responses. In OSA, hypoxemia caused by upper airway obstruction activates inflammatory pathways, leading to increased levels of C-reactive protein and interleukin-6, which in turn suppresses *NOS3* expression and increases the risk of ischemic stroke. Mikhail et al found that after 12 weeks of intensive CPAP treatment in OSA patients, there was no change in the *NOS3* gene enzyme, consistent with our TWAS analysis,[40] which showed no significant association between *NOS3* gene expression and OSA. We speculate that the role of the *NOS3* gene in the pathological mechanisms of OSA may require the involvement of other genes. In future research, the *NOS3* gene could potentially serve as a biomarker for predicting and identifying individuals at high risk of stroke within the OSA population early on. This could aid in the development of personalized prevention and intervention strategies to reduce the likelihood of disease occurrence. *ATG9B* is referred to as Autophagy-related 9B. It is responsible for membrane trafficking and dynamic regulation during the formation of the autophagosome. Through this process, cells are able to degrade and recycle damaged or excess organelles and proteins, maintaining cellular homeostasis and survival. In ischemic brain injury, knockout of the *ATG9A* gene can reduce neuroinflammatory responses, decrease brain edema and infarct volume, thereby providing protective effects against brain injury. And the role of *ATG9B* in OSA may be particularly significant due to its involvement in the regulation of autophagic flux during hypoxic stress.[41] However, current research has not clearly demonstrated a direct association between the *ATG9B* gene and ischemic stroke or OSA. In summary, intermittent hypoxia triggers systemic inflammation and oxidative damage in OSA, while in ischemic stroke, acute oxidative stress and neuroinflammatory responses contribute to tissue damage and functional deficits. The pleiotropic effects of the *NOS3* and *ATG9B* genes may contribute to the development and progression of OSA and ischemic stroke by regulating vascular function and autophagy mechanisms. This further highlights their significance as potential therapeutic targets.

In the result of TWAS, *POLR3H* and *TRMO* exhibited the most widespread expression across tissues. Previous studies have not identified a direct association between them and OSA or ischemic stroke. *EFCAB13* and *RALGAPA1*, which were the genes with the second-most widespread tissue expression, were found to have significant correlations with various conditions. Chen et al discovered that mRNA expression levels of *EFCAB13* were significantly associated with low-density lipoprotein C (LDL-C).[42] Additionally, the upregulation of *EFCAB13* has been linked to hypomethylation in heart failure.[43] LDL levels are positively correlated with the severity of OSA.[44] Previous research has found that patients with OSA experience repetitive hypoxic events during sleep, which may lead to increased oxidative stress. This stress environment makes LDL more susceptible to oxidation, forming oxidized LDL (oxLDL), which is a key factor in the development of atherosclerosis and significantly increases the risk of cardiovascular disease.[45] Consistent with this, alterations in LDL levels due to *EFCAB13* variants may contribute to the co-occurrence of OSA and ischemic stroke. Additionally, Chang et al identified *RALGAPA1* as a risk locus in the Taiwanese population.[46] This suggests that the role of lipid metabolism in the complex interplay between these two diseases warrants further investigation in future studies. This study did not identify a positive causal relationship between OSA and stroke through MR analysis, However, using various post-GWAS analysis methods, we successfully identified pleiotropic factors shared between these traits. These findings suggest that the genetic overlap between OSA and stroke is primarily driven by pleiotropic effects rather than direct causation.

This study has several notable strengths, particularly its use of multi-omics approaches to explore the genetic overlap between OSA and ischemic stroke. To account for differences in genetic backgrounds across ethnic groups, we focused

on European populations for both conditions and selected GWAS datasets with sample sizes exceeding 5000, enhancing the robustness and reliability of our findings.

However, the study has certain limitations. First, it exclusively included ischemic stroke traits, as ischemic stroke represents the majority of stroke cases. This focus may limit the applicability of our findings to other subtypes, such as hemorrhagic stroke. Future studies should consider incorporating GWAS data for hemorrhagic stroke as such datasets become increasingly available, enabling more comprehensive classification analyses. Second, the GWAS data used were derived solely from European populations, which may constrain the generalizability of our findings to non-European populations. Genetic diversity across ancestries can lead to variations in gene-disease associations, and the lack of representation from diverse populations underscores the need for future research involving more globally representative datasets. Lastly, there is a possibility that the reported pleiotropic genes reflect confounding effects, which may vary across different population strata. These genes could influence intermediate traits that indirectly affect both conditions, rather than having direct effects on the diseases themselves. Given the genetic diversity across populations, the strength and nature of these indirect effects may differ, emphasizing the importance of considering population stratification in such analyses. Although we employed functional annotation tools and gene-level analyses to mitigate this risk, these methods cannot fully account for population-specific genetic variations or eliminate confounding. Future studies incorporating diverse population datasets, experimental approaches, and advanced causal inference models will be necessary to disentangle these indirect effects, better understand the role of population stratification, and further validate our findings.

Conclusions

This study identified a significant positive genetic correlation between OSA and ischemic stroke and further explored their shared genetic architecture from multiple perspectives. The analysis highlighted the role of genes associated with inflammation and endothelial cell function in the comorbidity of these conditions. These findings provide valuable genetic insights into the complex relationship between OSA and ischemic stroke, offering a foundation for future research into their underlying mechanisms.

Ethics Approval

The ethics committee of Fujian University of Traditional Chinese Medicine strictly adheres to the Declaration of Helsinki and the International Ethical Guidelines for Health-related Research Involving Humans. The present study uses legally obtained publicly available data, meeting the conditions for exemption from review as stated in the Measures for Ethical Review Methods for Life Sciences and Medical Research Involving Humans.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no potential conflicts of interest relevant to this article.

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