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ORIGINAL RESEARCH

Triangular Causality Among Pulmonary Hypertension, Sleep Disorders, and Brain Structure at the Genetic Level: A Mendelian Randomization Study Focused on the Lung-Brain Axis

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Background: The bidirectional relationship between pulmonary hypertension (PH) and sleep disorders has attracted significant research attention. The concept of the lung-brain axis has further highlighted the need for a holistic approach to managing these diseases.

Methods: This study used bidirectional two-sample Mendelian Randomization (MR) to explore the genetic-level causal relationships between PH, sleep disorders, and structural brain changes. GWAS data for PH were pooled from four cohorts; data on four sleep disorder subtypes were sourced from the FinnGen database; and data on 15 structural brain changes were obtained from the ENIGMA Consortium. To ensure reliability, we applied strict data selection, multiple corrections, heterogeneity assessments, and sensitivity tests. Visualizations included forest plots, scatter plots, funnel plots, and leave-one-out plots.

Results: MR analysis revealed a significant causal relationship between PH and both obstructive sleep apnea (OSA) (OR = 1.022, 95% CI = 1.006–1.039, P = 0.006, PBonferroni = 0.025) and general sleep disorders (OR = 1.018, 95% CI = 1.003–1.033, P = 0.018, PFDR = 0.036), with no evidence of reverse causation and multivariable MR analyses also demonstrated significant results. PH was linked to changes in total brain volume (P = 0.032) and cerebral white matter (P = 0.035). Amygdala changes appeared to reduce the risk of sleep disorders (P = 0.008) and OSA (P = 0.014). Sensitivity analyses showed no heterogeneity, pleiotropy, or significant outliers.

Conclusion: This study identifies significant causal links between PH, sleep disorders, and structural brain changes, establishing a triangular cyclic relationship that supports the lung-brain axis concept. These findings inform clinical management of PH and its comorbidities.

Keywords: pulmonary hypertension, sleep disorders, obstructive sleep apnea, brain structure, lung-brain axis, genetic associations

Introduction

Pulmonary hypertension (PH) is a rare and complex disease affecting the pulmonary circulation, characterized by increased pulmonary vascular resistance and elevated pressure in the pulmonary artery.[1] PH is classified into five categories: PH caused by pulmonary vascular disease; PH resulting from left heart disease; PH associated with lung disease or hypoxia; PH due to chronic thromboembolic disease; and PH caused by various disorders, representing a miscellaneous collection of syndromes.[2] According to the 2022 ESC/ERS Guidelines, PH affects 1% of the global population, with the UK experiencing a doubling in prevalence over the last decade, alongside a global increase in the disease's incidence as the average age of populations rises.[3] Additionally, data from the PH Registry indicates that the 1-year survival rate for PH patients varies between 68% and 93%, while the 3-year survival rate ranges from 39% to

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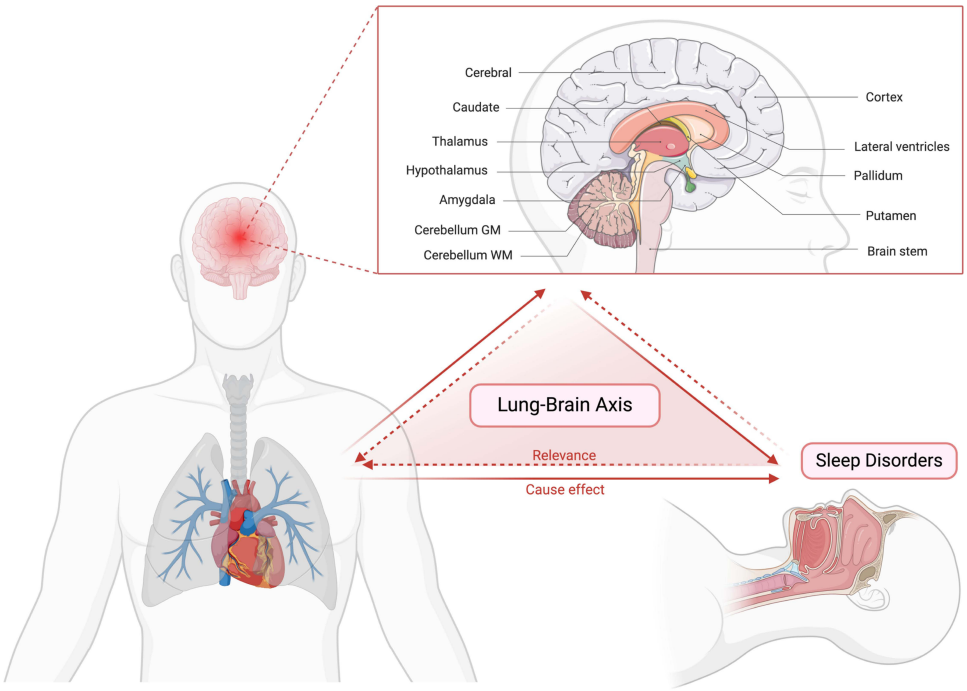
Graphical Abstract



77%.[4] It also leads to systemic complications including sleep disorders, pulmonary encephalopathy, heart failure, and chronic kidney disease, as the disease affects both the respiratory and circulatory systems, significantly impacting the patient's health and quality of life.[5–7]

The primary types of sleep disorders are insomnia, narcolepsy, and sleep apnea, with obstructive sleep apnea (OSA) being the most prevalent, affecting approximately 1 billion individuals globally.[8] Characterized by intermittent shallow or interrupted breathing due to repeated partial or complete blockage of the upper airway during sleep, this condition leads to lower oxygen levels and disrupted sleep patterns, ultimately impairing sleep quality and daytime functionality.[9,10] Although the underlying causes and mechanisms differ, the bidirectional relationship between OSA and PH has sparked considerable interest among researchers. Previous studies have long identified sleep-disordered breathing (SDB) as a cause of category 3 PH.[11] SDB is also more common in pulmonary vascular diseases such as PAH and CTEPH, likely due to increased ventilation-perfusion mismatch and a higher frequency of obstructive and central respiratory events during sleep.[11,12] The pathophysiological mechanisms of obstructive sleep apnea (OSA) in PH are still under active investigation, with current research focusing on intermittent hypoxia, negative pleural pressure, and endothelial dysfunction.[13–15] Similarly, hypoxia, mechanical factors due to increased inspiratory effort, and reflex mechanisms that directly affect the vascular system are the primary mechanisms potentially responsible for the observed increase in pulmonary artery pressure associated with obstructive sleep apnea.[5,16,17] Existing research shows that the prevalence of PH in patients diagnosed with OSA varies significantly, ranging from 17% to 70%,[18,19] and studies investigating the prevalence of OSA among patients with a known history of PH are even rarer.[5] The variability in results across studies can be attributed to the heterogeneity of the disease, variations in clinical definitions used, and the small sample sizes involved.[20] Therefore, actively employing new methods and strategies to investigate the potential interactions between the two offers a highly practical approach for clinical application.

The concept of the "Lung-Brain Axis" revolves around the physiological and pathological connections between the lungs and the brain, encompassing the systems of interaction and communication between them. Beyond being a site for gas exchange, the lungs also influence brain function both directly and indirectly through blood oxygenation levels and

inflammatory factors, with reciprocal effects from the brain. In our comprehensive study, we found that both diseases associated with these organs can significantly impact brain function, particularly manifesting in cognitive impairments and psychiatric disorders. Enhanced understanding of lung-brain interactions has invigorated efforts to develop more comprehensive treatment approaches. For instance, pulmonary artery denervation has been shown to significantly reduce PH and remodel the pulmonary artery, while cutting the vagus nerve's connection to the pulmonary region can lead to fibrosis in the pulmonary arteries and airways.[21] Data from Christopher Caleb Angelakos et al indicate that a group of neuropeptide S neurons, along with their subpopulations, can function in the brain to enhance arousal, thereby regulating both respiration and arousal levels.[22]

Genome-wide association studies (GWAS) offer new insights by analyzing genetic variations at thousands of loci to identify genes associated with specific diseases, thereby helping researchers understand the genetic foundations of these conditions at a molecular level. Mendelian Randomization (MR) is a genetic epidemiological method that uses genetic variants associated with diseases, identified through GWAS, as instrumental variables. This approach assesses the causal relationships between exposures (such as lifestyle habits and biomarkers) and specific health outcomes. Leveraging the principle that genetic variants are fixed and randomly assigned at conception, Mendelian Randomization (MR) serves as a robust alternative to traditional observational studies, providing a high level of evidence-based validation akin to randomized controlled trials. Simultaneously, Mendelian Randomization (MR) analysis operates at the genetic level, enhancing existing studies by significantly reducing the impact of confounding factors and effectively minimizing the potential for reverse causality. In this study, utilizing phenotypic GWAS pooled data, we initially applied Mendelian Randomization (MR) analysis to establish the bidirectional associations and potential causality between PH and sleep disorder diseases at the genetic level. Subsequently, we investigated the genetic correlations between these disorders and the longitudinal changes in brain structure throughout the life cycle, aiming to further substantiate the existence of the lung-brain axis. Ultimately, our goal is to develop new guidelines for the clinical management, personalized diagnosis, and treatment of PH, sleep disorder diseases, and their associated neurological comorbidities.

Methods

Study Design

This study offers initial insights into the potential bidirectional causal link between PH and sleep disorder diseases using two-sample Mendelian randomization analysis, exploring whether they collectively contribute to longitudinal changes in brain structure, thereby supporting the concept of the lung-brain axis. To ensure the scientific validity of the findings, it is imperative to satisfy the three fundamental assumptions of Two Sample Mendelian Randomization (TSMR).[23] The chosen Instrumental variable (IV) should exhibit a strong correlation with the exposure in order to mitigate endogeneity issues. Once again, this is to ensure that the IV is independent and not associated with identified protective and risk factors. The last consideration is to ensure that the IV exclusively impacts phenotypes of outcomes through exposure factors. The Figure 1 provided below offers a clear overview of the details involved in our study.

Data Resource

Original GWAS Data Source for PH and OSA

Pooled GWAS data on PH outcomes were sourced from four international case-control study cohorts, representing multicenter sites across the United States, France, Germany, the Netherlands, the United Kingdom, and Italy. These include the National Institute for Health Research Biological Resources Rare Diseases Study (NIHRBR) in the UK, the US Pulmonary Arterial Hypertension Biospecimen and Data Repository/PAH Biorepository (PAHB) Study in the US, the Paris Pulmonary Hypertension Alleles Associated Risk Cohort (PHAAR) study, and the British Heart Foundation Pulmonary Hypertension GWAS (BHFPAH). The cohorts involved a total of 11,744 individuals of European ancestry, which included 2,085 patients. PH was defined using hemodynamic criteria from international guidelines, including only unrelated individuals with idiopathic, hereditary, or anorexia-related PH, while excluding those with other known causes of the condition. The study tested individuals from the NIHRBR, PHAAR, and BHFPAH cohorts to ensure that identical or related individuals were not included in the analysis. Further quality control steps are detailed in Supplementary Material 1 and the study by Christopher

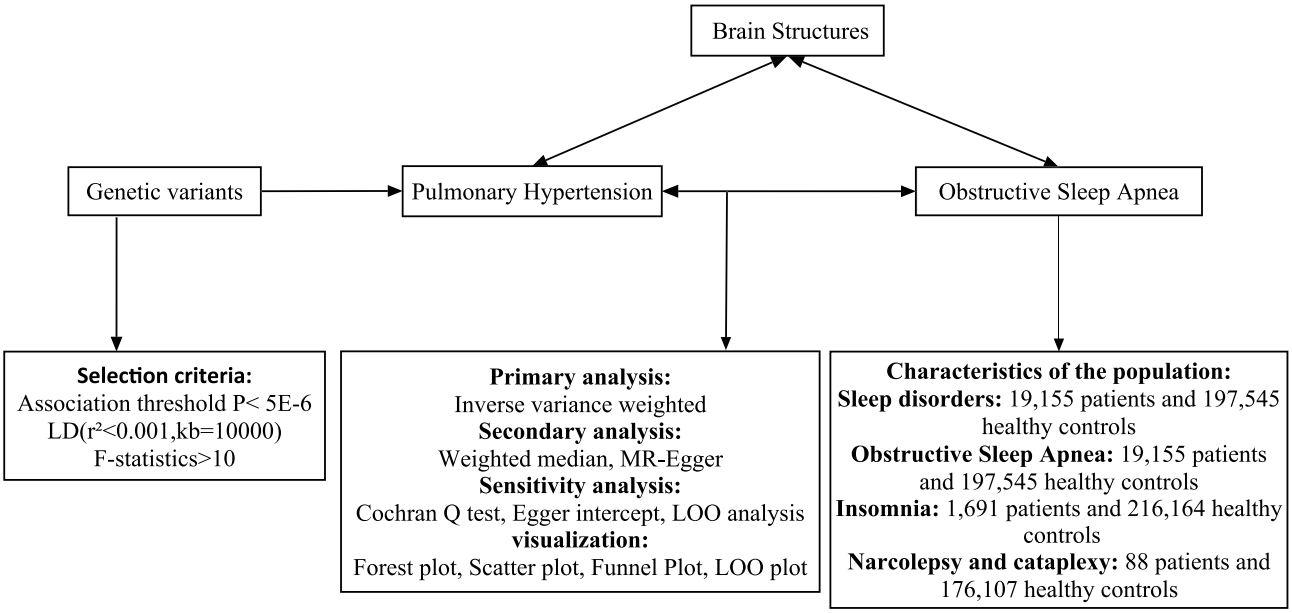


Figure 1 Study Design for MR Analysis of PH, sleep disorders and structural brain changes.
Abbreviations: MR, Mendelian randomization; PH, Pulmonary Hypertension; LD, Linkage Disequilibrium; LLO, Leave-One-Out.

J. Rhodes et al.[24] The population characteristics of the four independent PH studies are specifically outlined in the Table S1. On April 18, 2024, we retrieved GWAS summary data for PH from the NHGRI-EBI GWAS Catalog, under project number GCST007228, accessible at https://www.ebi.ac.uk/gwas/.[24]

To avoid the potential issues of sample selection bias and confounders in genetic associations that could affect MR results, we selected the Finnish cohort (FinnGen Biobank), which shares the same European ancestry. Launched in 2017, the FinnGen biospecimen repository utilizes data from the National Health Registry to delve into genetic information, aiming to uncover the genetic foundations of diseases and pinpoint new therapeutic targets and diagnostic methods. In selecting outcomes for sleep disorders, our study primarily focused on the four most prevalent subtypes: sleep disorders (ICD10: F51, G47), obstructive sleep apnea (ICD10: G47.3), Narcolepsy and cataplexy (ICD10: F51.0, G47.0), and narcolepsy (ICD10: G47.4) (Table S2).[25] Databases categorize diseases using ICD codes, and relying on clinical diagnoses may introduce biases, such as underreporting or misclassification of less common or milder conditions.

Sources of GWAS Data for Brain Structure

Data on genetic variations associated with longitudinal changes in brain structure were sourced from a multicenter study, with key contributors including the NeuroImaging Genetics through Meta-Analysis (ENIGMA) Consortium, the Alzheimer's Disease Neuroimaging Initiative (ADNI), 1000BRAINS, and other collaborators.[26] In this GWAS meta-analysis, data from 40 longitudinal cohorts (15,640 all-age participants) were consolidated. Following a coordinated two-stage analysis, a set of 15 metrics were ultimately considered, including eight global metrics (such as whole brain excluding the brainstem but including the cerebellum, mean cortical thickness, surface area measured at grey-white matter boundaries, cortical and cerebellar grey- and white-matter volumes, and total lateral ventricle volumes) and seven subcortical metrics (including caudate, thalamus, chiasmatic nuclei, hippocampus, pallidum, nucleus accumbens, and amygdala). The population characteristics of these 40 cohorts are provided in the Table S3. The study conducted thorough sensitivity analyses by repeating SNP and genetic evaluations across several subgroups: (1) adding four non-European or mixed ancestry cohorts (n = 540, total n = 15,640); (2) excluding cohorts that did not meet the minimum sample size (n > 75) or scanning interval (≥ 0.5 years), leaving n = 14,601; (3) removing cohorts from each diagnostic group, resulting in n = 13,390; and (4) adjusting for disease status as a covariate. Effect sizes for SNPs were consistently similar across all subgroups, both in SNP-based and gene-based analyses. Additionally, results remained comparable in the healthy subgroups and when correcting for disease status.[26]

Similarly, All data sources on PH, sleep disorders and brain structure utilize tables to display the characteristics of the data.

Instrumental Variable Selection

To address the first core hypothesis, we initially screened for exposure-related instrumental variables (IVs) by setting a significance threshold of P<5E-6, ensuring an adequate number of SNPs for further analysis. Due to limited data on genetic variations related to brain structure, this threshold was expanded to P<1E-5. Setting a minor allele frequency (MAF) threshold of 0.01 for target variants ensures that the genetic variants employed possess sufficient statistical power.[27] To ensure the independence of the IVs, we set a distance threshold of $R^2 < 0.01$ and 10,000 kb to address the issue of Linkage Disequilibrium (LD), which refers to the non-random association between genetic loci. During the screening process, SNPs with palindromic structures were also excluded to minimize their potential impact on random assignment. Finally, we computed the F-statistic ($F=R^2\times(N-2)/(1-R^2)$ and $R^2=2\times(1-EAF)\times EAF\times\beta^2$, R^2) for the selected instrumental variable to ensure its value exceeds 10, aiming to mitigate the influence of weak instrumental variables.[23] Furthermore, to ensure the accurate selection of instrumental variables, we utilized the FUMA tool to map genes and subsequently performed enrichment analysis of associated biological pathways (https://fuma.ctglab.nl/).

Bidirectional TSMR Analysis

In this study, we initially selected three sets of positive exposure-outcome pairs: PH-OSA, PH-brain structure, and OSA-brain structure. In the event of a statistically significant finding, we will implement reverse validation for the positive results to rule out any potential effects of reverse causation. In some cases, certain instrumental variables were represented by a single SNP, and in such instances, the Wald ratio method was employed to evaluate causal effects. Moreover, when multiple SNPs are present in the IV, the Inverse Variance Weighting (IVW) method is the most frequently utilized approach for MR analysis.[28] This method amalgamates the Wald ratios through meta-analysis principles to provide an overall estimate of the causal effect. To reduce the impact of potential heterogeneity, the IVW fixed-effects model was used for non-significant p-values (> 0.05), while the random-effects model was applied for significant p-values (< 0.05). The weighted median method and the MR-Egger method are commonly employed to complement the primary analysis, ensuring consistency in result directionality and providing robust outcomes when addressing potential horizontal pleiotropy in IVs.[29] To strengthen the robustness of our findings, we separately calculated the statistical power for dichotomous and continuous outcome variables using the given equation: Power = Φ(β * sqrt(R² * (n_case * n_control)/n) - Z_(1-α/2)) and Power = Φ((β * sqrt(R² * n)/σ_Y) - Z_(1-α/2)). where Φ is the cumulative distribution function of the standard normal distribution, β is the effect estimate, R² is the variance explained by the instrumental variable, n_case and n_control are the sample sizes of cases and controls, n is the total sample size, σ_Y is the standard deviation of the outcome variable and Z is the critical value for significance level α.[30] Furthermore, we applied Steiger filtering to confirm the correct direction of associations between exposure and outcome phenotypes (Steiger P > 0.05).

Sensitivity Analyses

This study incorporates multiple sensitivity analyses to validate and rectify positive MR findings through various methods. To assess possible heterogeneity, we employed Cochran's Q statistic to present whether its significance, meanwhile Leave-One-Out (LOO) analysis was directly visualised to test for the presence of anomalous SNPs that significantly affected the results (a significance threshold of p<0.05 was applied).[31] In assessing the potential presence of horizontal pleiotropy, the magnitude of the intercept in the Egger regression signifies the significance of the average pleiotropic effect among all IVs.[29] Additionally, Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) aims to enhance the robustness of results by systematically detecting and excluding instrumental variables one by one, thereby mitigating the impact of pleiotropy.[32] To account for the potential confounding effects of risk factors for sleep disorders and environmental influences, multivariate Mendelian randomization (MVMR) analyses were conducted, incorporating factors such as noise pollution (ukb-b-10325), PM2.5 (ukb-b-10817), and smoking (ieu-b-4877). These

analyses aimed to evaluate whether these factors could impact the causal relationship between exposure and outcome. The study primarily employed a basic model for this assessment.

The code employed in this study was executed in the R 4.4.0 environment, utilizing primarily the R packages for TwoSampleMR and MR-PRESSO. Bonferroni correction and False Discovery Rate (FDR) were applied to multiple tests, with P<0.05 considered statistically significant.

Result

Potential Causal Relationship Between PH and OSA

Following a meticulous quality control process, we established a screening threshold of P<5E-06 for selecting exposed phenotypic SNPs and ultimately identified 24 instrumental variables strongly associated with PH. The mean value of the F-statistic is 221.75, significantly mitigating the potential impact of weak instrumental variables (Table S4). Using the FUMA tool, these instrumental SNPs were mapped to 18 key genes, which were found to be enriched in pathways related to calcium-mediated signaling and inorganic cation transmembrane transport, both of which are strongly linked to the physiological mechanisms of PH (Table S5). For the accuracy and interpretation of genetic analysis, rs2409744 was excluded due to its palindromic structure. Using the Inverse Variance Weighted (IVW) approach as the primary method, we confirmed a significant causal relationship between PH and two of the four sleep disorder endpoints. The current study indicates that the occurrence of PH significantly increases the risk of sleep disorders (OR=1.018, 95% CI=1.003–1.033, P=0.018, P_{FDR}=0.036), including obstructive sleep apnea (OR=1.022, 95% CI=1.006–1.039, P=0.006, $P_{Bonferroni}$= 0.025). It is evident that after applying the Bonferroni correction, PH remains significantly causally related to OSA, and following the FDR multiple correction, PH is causally related to both OSA and sleep disorders. Additionally, both the MR Egger (OR=1.027, 95% CI=1.006–1.049, P=0.021) and Weighted Median methods (OR=1.024, 95% CI=1.001–1.047, P=0.018) indicate a unidirectional causal relationship between PH and OSA (Figure 2 and Table 1). The funnel plots displayed a roughly symmetrical trend, suggesting that the selected instrumental variables consistently influenced the exposure variables, thereby enhancing the robustness of the results. In the heterogeneity tests, the Cochran's Q statistic values for both IVW and MR-Egger were all above 0.088, and none of the leave-one-out (LOO) analyses and forest plots indicated the presence of anomalous SNPs (Figure 3 and Figure S1). Additionally, the intercept values from Egger's regression were all above 0.05 for the positive results. Moreover, the MR-PRESSO method did not

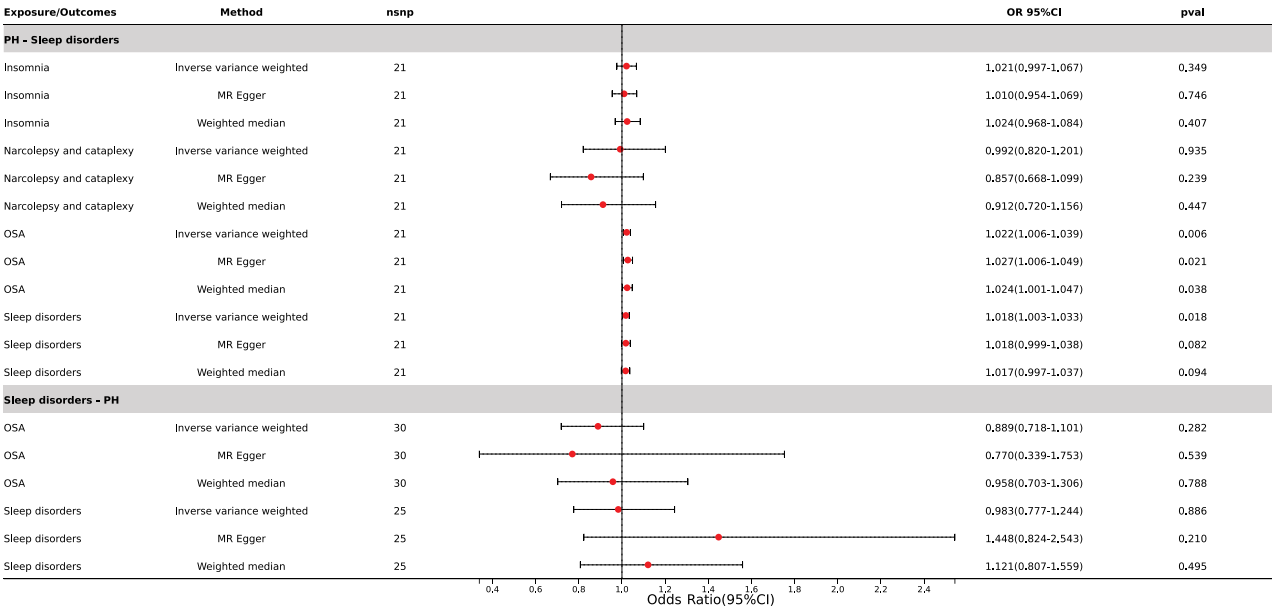


Figure 2 Associations of genetically predicted PH, sleep disorders and structural brain changes in Mendelian randomization analysis.
Abbreviations: PH: pulmonary hypertension; OSA: Obstructive Sleep Apnea; OR: Odds Ratio.

Table 1 Details of the Bidirectional Causal Relationship Between Pulmonary Hypertension and Sleep Disorders

Exposure/Outcomes	Method	nsnp	or	or_lci95	or_uci95	OR 95% CI	Pval	FDR	Bonferroni	Power
PH - Sleep disorders										
Insomnia	**Inverse variance weighted**	21	1.021	0.977	1.067	1.021(0.997–1.067)	0.349	0.465	1	10%
	MR Egger	21	1.010	0.954	1.069	1.010(0.954–1.069)	0.746	0.746	1	5.10%
	Weighted median	21	1.024	0.968	1.084	1.024(0.968–1.084)	0.407	0.447	1	11.70%
Narcolepsy and cataplexy	**Inverse variance weighted**	21	0.992	0.820	1.201	0.992(0.820–1.201)	0.935	0.935	1	2.90%
	MR Egger	21	0.857	0.668	1.099	0.857(0.668–1.099)	0.239	0.318	0.956	20.90%
	Weighted median	21	0.912	0.720	1.156	0.912(0.720–1.156)	0.447	0.447	1	10.10%
Obstructive Sleep Apnea	**Inverse variance weighted**	21	1.022	1.006	1.039	1.022(1.006–1.039)	**0.006**	**0.024**	**0.024**	57.50%
	MR Egger	21	1.027	1.006	1.049	1.027(1.006–1.049)	0.021	0.084	0.084	74.90%
	Weighted median	21	1.024	1.001	1.047	1.024(1.001–1.047)	0.038	0.152	0.152	64.90%
Sleep disorders	**Inverse variance weighted**	21	1.018	1.003	1.033	1.018(1.003–1.033)	**0.018**	**0.036**	0.072	46.50%
	MR Egger	21	1.018	0.999	1.038	1.018(0.999–1.038)	0.082	0.164	0.328	46.50%
	Weighted median	21	1.017	0.997	1.037	1.017(0.997–1.037)	0.094	0.188	0.376	42.40%
Sleep disorders - PH										
Obstructive Sleep Apnea	**Inverse variance weighted**	30	0.889	0.718	1.101	0.889(0.718–1.101)	0.282	0.564	0.564	35.80%
	MR Egger	30	0.770	0.339	1.753	0.770(0.339–1.753)	0.539	0.539	1	94.40%
	Weighted median	30	0.958	0.703	1.306	0.958(0.703–1.306)	0.788	0.788	1	8.40%
Sleep disorders	**Inverse variance weighted**	25	0.983	0.777	1.244	0.983(0.777–1.244)	0.886	0.886	1	4.20%
	MR Egger	25	1.448	0.824	2.543	1.448(0.824–2.543)	0.210	0.42	0.42	99.90%
	Weighted median	25	1.121	0.807	1.559	1.121(0.807–1.559)	0.495	0.788	0.99	34.10%

Notes: Pval, FDR, and Bonferroni: The three bolded columns indicate statistical significance.
Abbreviations: PH, Pulmonary hypertension; MR, Mendelian randomization; OR, Odds Ratio; SNP, Single Nucleotide Polymorphism; FDR, False Discovery Rate.

identify any outliers, effectively eliminating the impact of horizontal pleiotropy on the findings and ensuring the robustness of the results. The detailed results of the sensitivity analysis are presented in Table 2. The same screening conditions were used to validate reverse causality, we identified 32 (obstructive sleep apnea) and 25 (sleep disorder) SNPs as instrumental variables, respectively, excluding rs9318788 because it did not achieve an F-statistic value greater than 10 (Table S6). Unfortunately, no reverse causal relationship (Table S7) was found between obstructive sleep apnea (OR=0.889, 95% CI=0.718–1.101, P=0.281), sleep disorders (OR=0.983, 95% CI=0.777–1.244, P=0.886), and the risk of PH, further affirming the accuracy and robustness of the positive findings. In addition, Steiger filtering found no SNPs showing evidence of reverse causality, further indicating the validity of the results. The results of our multivariate Mendelian randomization analysis revealed that, after adjusting for the effects of noise, PM2.5, and smoking, PH remained significantly associated with OSA (OR = 1.020, 95% CI = 1.001–1.030, P = 1.33E-05) and sleep disorders (OR = 1.020, 95% CI = 1.011–1.030, P = 1.33E-05), indicating a robust causal relationship (Tables S8–S11).

Bidirectional Causality Between PH and Structural Changes in the Brain

The IVW method have identified two positive causal relationships between PH and age-independent longitudinal changes in brain structure throughout the lifespan. The study's findings indicated a potential causal relationship between the incidence of PH and changes in total brain (OR=7.03E+69, 95% CI=8.41E+05-5.88E+133, P=0.032) and cerebral white matter (OR=3.46E+32, 95% CI=2.09E+02-5.72E+62, P=0.035), with the direction suggesting that PH acts as a risk factor (Tables S12 and S13). In the sensitivity analyses, we found no significant heterogeneity or pleiotropy, and there were no outliers affecting the study's results (Tables S14, S15 and Figure 4). When analyzing brain structural changes as the exposure, we identified a total of 153 genetic variants as instrumental variables linked to 15 brain structures: amygdala (15 SNPs), caudate (7 SNPs), cerebellum gray matter (12 SNPs), cerebellum white matter (15 SNPs), cerebral WM (6 SNPs), cortical GM (11 SNPs), hippocampus (7 SNPs), lateral ventricles (12 SNPs), mean thickness (11 SNPs), pineal gland (8 SNPs), thalamus (8 SNPs), putamen (12 SNPs), surface area (6 SNPs), cerebellum (9 SNPs), and total brain (14 SNPs). However, when PH was considered as the outcome, we found no potential causal relationship with any specific brain structure change (Tables S16 and S17).

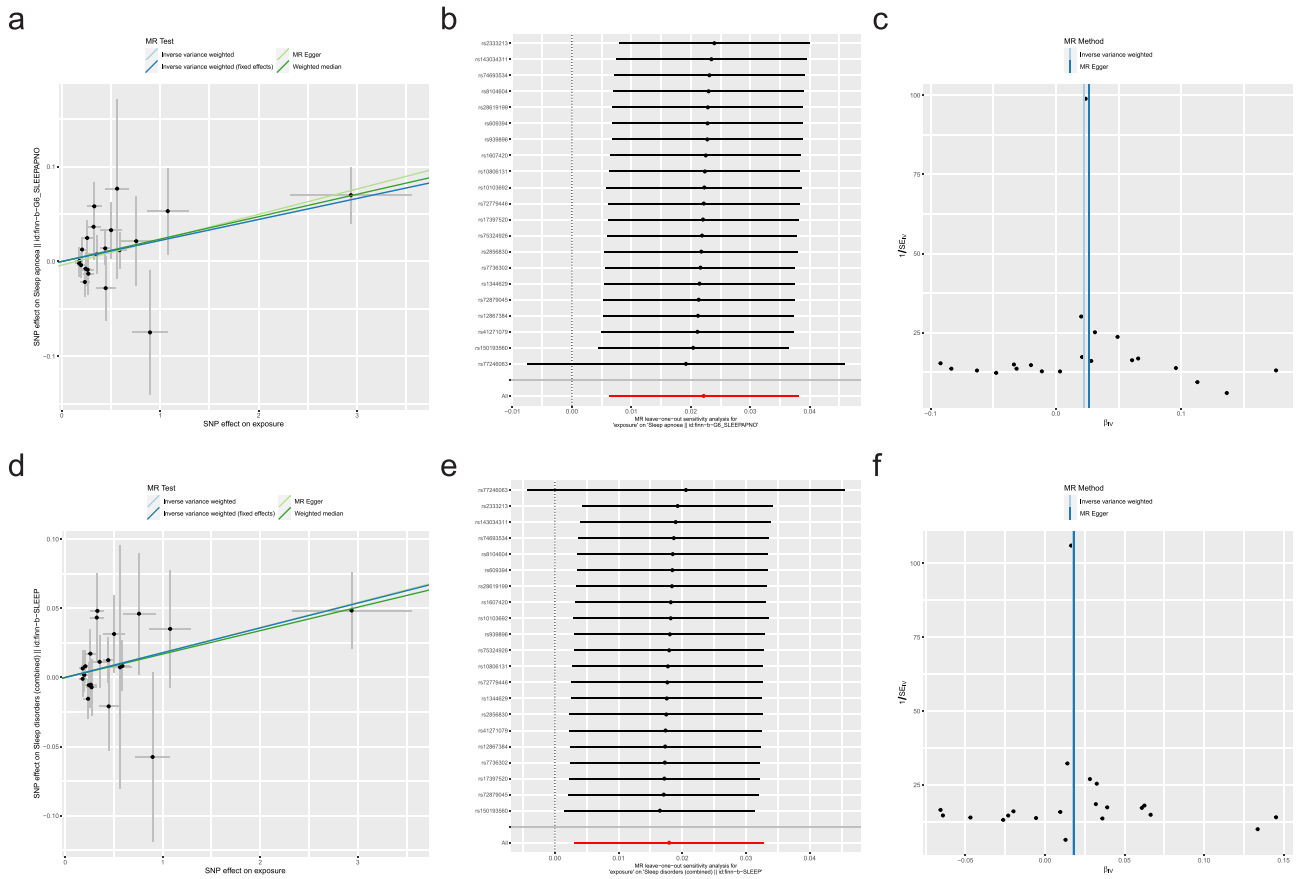


Figure 3 Visual graphs related to the sensitivity analysis of Pulmonary Hypertension associated with sleep disorders. (**a**) Scatter plot of Pulmonary Hypertension and Obstructive Sleep Apnea; (**b**) Leave-One-Out plot of Pulmonary Hypertension and Obstructive Sleep Apnea; (**c**) Funnel plot of Pulmonary Hypertension and Obstructive Sleep Apnea; (**d**) Scatter plot of Pulmonary Hypertension and Sleep disorders; (**e**) Leave-One-Out plot of Pulmonary Hypertension and Sleep disorders; (**f**) Funnel plot of Pulmonary Hypertension and Sleep disorders.

Bidirectional Causality Between Sleep Disorders and Structural Changes in the Brain

In the MR analysis of sleep disorders, the results indicate that the causal relationship primarily runs from structural changes in the brain to sleep disorders. Similarly, rs10475978, closely associated with OSA, was removed due to its palindromic structure. The findings indicate that surface area changing (OR=1.0003, 95% CI=1.00001–1.0006, P=0.044) is a risk factor for the development of obstructive sleep apnea (OSA) (Tables S18 and S19). Unfortunately, the results from the MR Egger method do not align with the directionality indicated by the IVW method, leading us to discount this positive finding. Conversely, changes in the amygdala seem to reduce the risk of OSA (OR=0.997, 95% CI=0.994–0.999, P=0.014) and sleep disorders (OR=0.996, 95% CI=0.994–0.999, P=0.008) favorably (Tables S20 and S21). Sensitivity

Table 2 MR Estimates and Sensitivity Analyses of the Causal Relationship Between PH and Sleep Disorders

Exposure/Outcomes	Heterogeneity p				MR-Egger Regression			MR-PRESSO Outliner Test		Steiger_pval
PH	**Q_IVW**	**Q_df_IVW**	**I^2**	**Q_pval_IVW**	**Intercept**	**SE**	**pval**	**Outliers**	**Global_test_p**	
OSA	16.669	20	−0.167	0.674	−0.004	0.006	0.506	NA	0.771	4.58E-154
Sleep disorders	12.117	20	−0.394	0.912	0.000	0.005	0.972	NA	0.956	8.49E-153
Insomnia	16.625	20	−0.169	0.677	0.010	0.016	0.543	NA	0.779	2.66E-152
Narcolepsy and cataplexy	13.220	20	−0.339	0.868	0.122	0.068	0.088	NA	0.817	6.08E-154

Abbreviations: PH, Pulmonary hypertension; OSA, Obstructive Sleep Apnea; MR, Mendelian randomization; SE, Standard Error; MR-PRESSO outliner, Mendelian Randomization Pleiotropy RESidual Sum and Outlier.

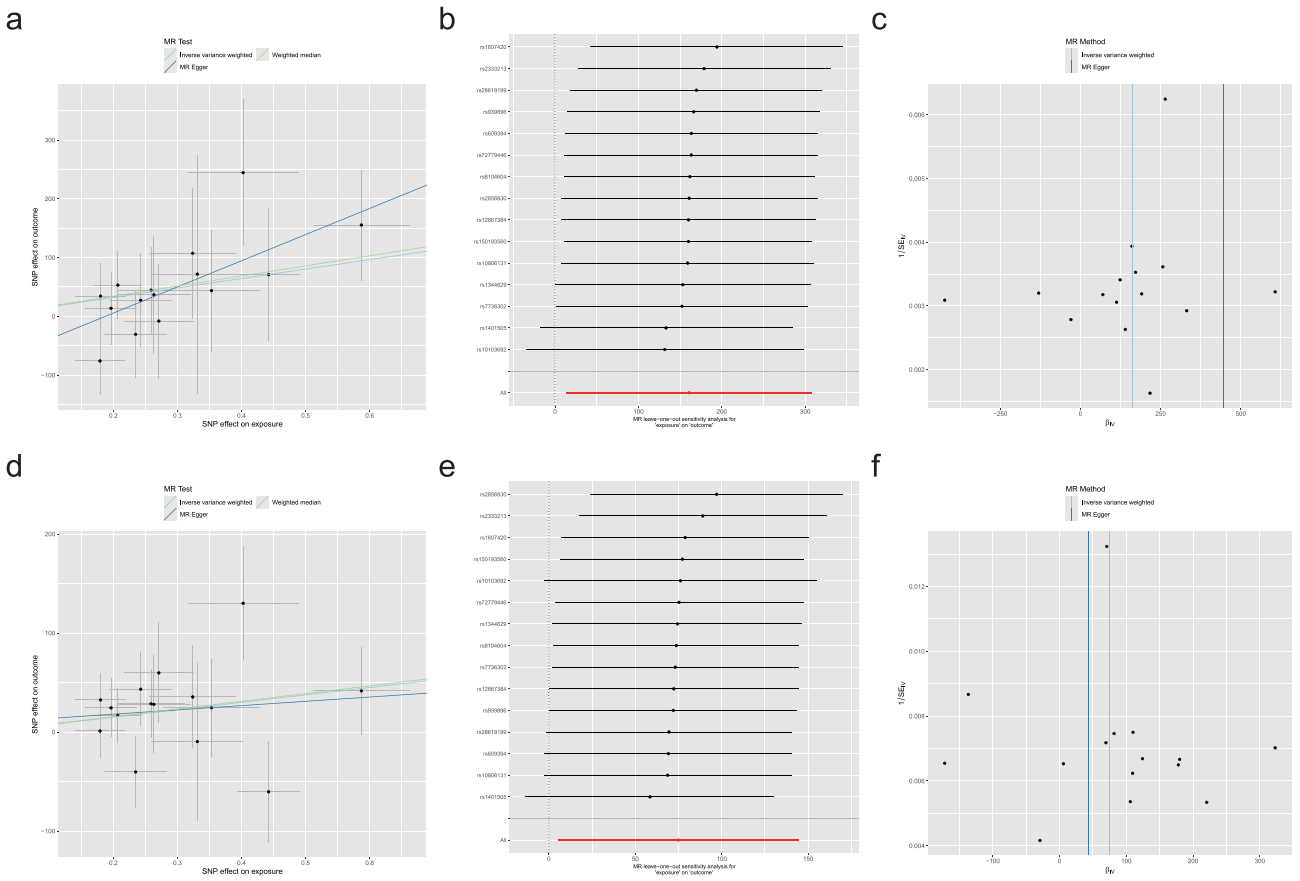


Figure 4 Visual graphs related to the sensitivity analysis of Pulmonary Hypertension with Brain structure associated. (**a**) Scatter plot of total brain and Pulmonary Hypertension; (**b**) Leave-One-Out plot of total brain and Pulmonary Hypertension; (**c**) Funnel plot of total brain and Pulmonary Hypertension; (**d**) Scatter plot of cerebral white matter and Pulmonary Hypertension; (**e**) Leave-One-Out plot of cerebral white matter and Pulmonary Hypertension; (**f**) Funnel plot of cerebral white matter and Pulmonary Hypertension.

analyses conducted on the additional material, with p-values greater than 0.05, confirmed the accuracy of the results. For the visualized images, the funnel plots appeared roughly symmetrical, the scatter plots showed a consistent trend, and the leave-one-out analysis did not detect any outlier instrumental variables that significantly affected the results (Figure 5). The MR-PRESSO analyses did not identify any outliers among the instrumental variables representing structural changes in the brain.

Discussion

This study represents the first attempt to establish a causal relationship between Pulmonary hypertension (PH), sleep disorders, and structural changes in the brain through Mendelian Randomization (MR) analysis at the genetic level. It aims to offer a new strategy for diagnosing and treating these conditions based on the lung-brain axis. The results showed a significant genetic correlation between PH and OSA, sleep disorder, but the reverse analysis did not reveal a similar directionality. After investigating the bidirectional causality between the disease and structural brain alterations, we observed a positive causal relationship of PH with total brain and cerebral white matter, and found that sleep disorders are more susceptible to structural brain alterations. Changes in the amygdala may lower the risk of both OSA and sleep disorders. Genetic associations among PH, sleep disorders, and brain structure appear to partially underpin the lung-brain axis.

The sequence of the onset of OSA and PH has been a subject of intense debate. OSA, as a unique physiological stressor, specifically contributes to the onset or progression of cardiovascular diseases, while the pulmonary circulation is also impacted by the intermittent hypoxic apnea associated with OSA.[5,19] The current study's results demonstrated

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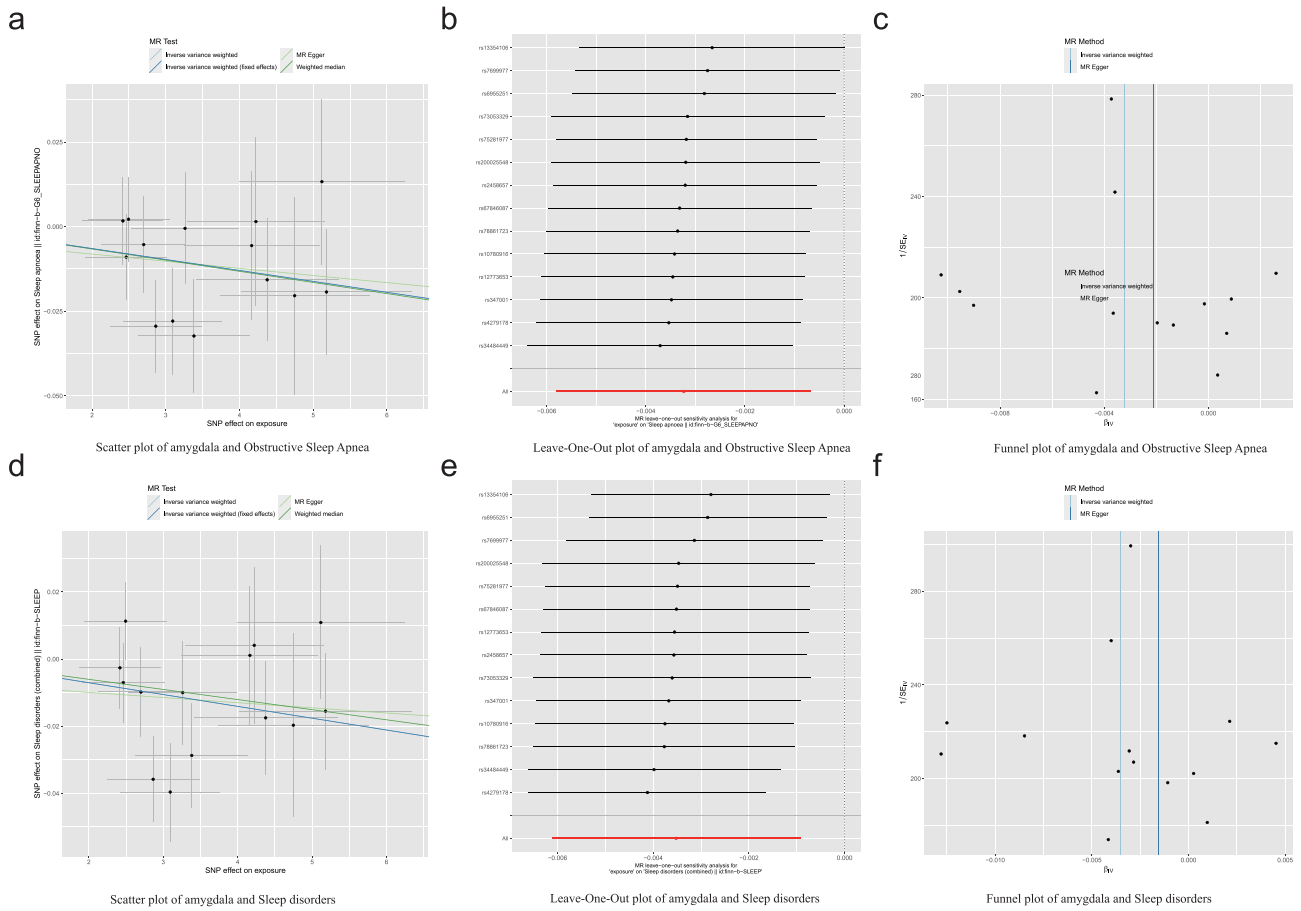

Figure 5 Visual graphs related to the sensitivity analysis of sleep disorders with Brain structure associated. (**a**) Scatter plot of amygdala and Obstructive Sleep Apnea; (**b**) Leave-One-Out plot of amygdala and Obstructive Sleep Apnea; (**c**) Funnel plot of amygdala and Obstructive Sleep Apnea; (**d**) Scatter plot of amygdala and Sleep disorders; (**e**) Leave-One-Out plot of amygdala and Sleep disorders; (**f**) Funnel plot of amygdala and Sleep disorders.

a significant causal relationship between PH and OSA, and PH was also causally linked to sleep disorders. However, the reverse Mendelian Randomization analysis did not show a genetic correlation between the two. This aligns with existing findings, where the prevalence of PH in patients with OSA varies widely, ranging from 15% to 80%, while the prevalence of OSA in patients with early-stage PH is higher, even up to 89%.[5,33,34] For this reason, given that OSA is a common sleep disorder, its occurrence in patients with PH might represent an association without causation or could add an additional burden. However, due to the rarity of PH, its presence in patients with OSA is unlikely to be coincidental, suggesting a potential causal relationship.[20]

Despite the disease's rarity, there are limited studies exploring whether PH can be a risk factor for OSA, making it an intriguing area for research. Patients with PH, particularly those showing symptoms of right heart failure, often suffer from fluid overload. This condition can lead to similar redistribution changes during sleep, exacerbating upper airway edema and OSA.[35,36] Consequently, reducing fluid retention and treating right heart failure may potentially have therapeutic effects on OSA.[37] Additionally, elevated pulmonary artery pressure in patients with PH stresses the right ventricle, causing right ventricular hypertrophy, dysfunction, and reduced cardiac output. This exacerbates the hypoxic and hypercapnic conditions associated with OSA, creating a vicious cycle that intensifies the severity of both OSA and PH.[20] In conclusion, it is crucial to focus more on the interaction between OSA and PH, and to explore the potential of CPAP, oxygen therapy, and other management strategies in treating these conditions and their associated comorbidities.[5] Simultaneously, little is known about how treating PH affects the occurrence and prognosis of OSA, highlighting the need for further research that could inform clinical management strategies for these conditions.

Observational studies have observed higher incidences of anxiety, depression, cognitive, and psychiatric disorders among individuals with PH, suggesting alterations in the brain structures and regions responsible for these functions.[38,39] This study identified a potential causal relationship where PH acts as a risk factor for changes in total brain and cerebral white matter. Roy et al discovered that individuals with PH exhibit altered brain structures, notably a significant reduction in gray matter volume in various regions involved in cognitive and emotional functions, including the hippocampus, insula, cerebellum, parahippocampal gyrus, temporal lobe, and frontal lobe.[40] In line with our findings, a patient with primary PH exhibited structural brain changes, primarily characterized by extensive cerebral white matter lesions.[41] We outline several potential mechanisms through which PH leads to structural changes in the brain, particularly affecting the white matter. Firstly, PH increases the pressure in the lung's blood vessels, impairing the lungs' capacity for gas exchange. Given that the brain is highly sensitive to oxygen, prolonged hypoxia can damage brain cells, particularly in the white matter regions. Furthermore, a systemic inflammatory state is commonly associated with PH, and inflammatory factors can cross the blood-brain barrier, impacting normal brain function. Microglia, the central nervous system's intrinsic innate immune cells, are highly active in a healthy brain and play a crucial role in influencing synaptic connectivity, activity, and neuronal health.[42] However, in animal models of PH, we observed a lack of microglia activation, which may also contribute significantly to neurodegenerative diseases and neuronal dysfunction.[43]

The amygdala is a small neural structure situated in the anterior portion of the temporal lobes on both sides of the brain, playing a crucial role in emotional processing and in the context of neuropsychiatric and neurodevelopmental disorders.[44] The study revealed that pediatric patients with OSA had greater amygdala volumes compared to healthy controls, and that amygdala volume was also associated with levels of anxiety and depression in children.[45,46] In another study, male patients with severe obstructive sleep apnea exhibited complex resting-state functional connectivity patterns in the amygdala subregion, which showed changes following six months of continuous positive airway pressure ventilation therapy.[47,48] The study did not establish a causal link between sleep disorders and structural changes in the brain; however, it unexpectedly revealed that changes in the amygdala may reduce the risk of disease. Although direct evidence supporting our results is lacking, several potential mechanisms may explain them. One of the initial and crucial insights is that the amygdala plays a key role in processing emotional responses, particularly emotions like fear and anxiety, which are risk factors for developing sleep disorders.[49,50] Changes in the amygdala may contribute to homeostatic sleep by regulating these emotional responses. Elevated muscular vasoconstrictor drive is commonly observed in patients with OSA, and this abnormal behavior may stem from functional changes in brain regions.[51] The right and left amygdala, to some extent, have been implicated in regulating sympathetic outflow through the brainstem, either directly or indirectly.[52]

This study offers multiple advantages and has significant clinical practical implications. Firstly, regarding the choice of data, we selected samples of European origin with exposures and outcomes from various countries. This approach not only minimizes the effect of population overlap but also aids in generalizing the study's findings across continental Europe. Furthermore, this study is the first to identify a triangular cyclic relationship between PH, sleep disorders, and structural changes in the brain through MR analysis. This provides insights for clinical diagnosis and individualized disease management based on the lung-brain axis. Additionally, this study aimed to demonstrate that while the occurrence of OSA in PH tends to be causal, PH in OSA appears more correlative, suggesting that this bidirectionality warrants further isolation in future research.

The main limitation of this study lies in the GWAS data sources for PH. The present study applied a relaxed threshold of $p<5E-06$ due to the limited number of PH-related IVs available under the conventional $p<5E-08$ criterion. However, this adjustment may have introduced weak instrument bias. In addition, although BMI is a recognized confounder in both PH and sleep disorders, it was not included in the analysis due to the insufficient number of SNPs associated with multiple exposure phenotypes in the MVMR analysis. As a result, potential confounding effects could not be fully accounted for, potentially impacting the causal interpretation. To enhance the study's robustness, future research should leverage larger GWAS datasets, implement stricter IV selection thresholds, and systematically consider key confounders such as BMI to refine the understanding of the PH-sleep disorder relationship. In addition, we were unable to examine the relationship between PH and sleep disorders in gender subgroups, nor could we explore the associations between the five PH subtypes and sleep disorders in greater depth. We observed smaller confidence intervals for OSA and sleep disorders,

likely due to the large patient sample size and stable number of healthy controls, resulting in smaller standard errors. To meet the design requirements of the Two-Sample Mendelian Randomization (TSMR), our study was restricted to populations from seven European-origin countries. To enhance the generalizability of our conclusions, we should consider expanding our research to include other populations, allowing us to observe similarities and differences in the results and to analyze potential causes. Furthermore, this MR analysis only establishes causal relationships between exposures and outcomes at the genetic level, using a computational model that provides statistical insights. However, the deeper underlying mechanisms still need exploration. To apply these results to clinical practice, extensive trials and observational clinical studies are necessary to ensure a robust and conclusive framework.

Conclusion

In this study, we have for the first time identified a significant causal association between PH and obstructive sleep apnea OSA, as well as other sleep disorders at the genetic level, using MR analysis. We also discovered potential causal links between PH and changes in total brain and cerebral white matter, as well as between the amygdala and both OSA and sleep disorders. The ultimate aim is to establish a lung-brain axis that could inform clinical management strategies for the comorbidities associated with PH and sleep disorders.

Data Sharing Statement

The analyses conducted for this article utilized data sourced from public databases (IEU Open GWAS Project: https://gwas.mrcieu.ac.uk/, FinnGen Biobank: https://www.finngen.fi/en/, ENIGMA: https://enigma.ini.usc.edu/research/ and GWAS Catalog: https://www.ebi.ac.uk/gwas/), the characteristics of the data are thoroughly detailed in the section on our data sources.

Ethical Approval

The GWAS summary data for pulmonary hypertension (PH), sleep disorders, and brain structures utilized in this study were sourced from publicly available databases. Consequently, the Institutional Review Board (IRB) of the First Hospital of Shanxi Medical University waived the need for IRB review and participant informed consent for this study.

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Author Contributions

Chenwei Zhang, Xuesen Su, and Yukai Zhang contributed to conception, design, acquisition, analysis, interpretation, drafted manuscript. Peiyun He contributed to acquisition, analysis, interpretation, drafted manuscript. ZhenXia Zhang and Xiaomei Kong contributed to analysis, interpretation, drafted manuscript. Yangyang Wei and Yiwei Shi contributed to conception and design, critically revised manuscript. 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. Reviewed and agreed on all versions of the article before submission, during revision, the final version accepted for publication, and any significant changes introduced at the proofing stage.

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The authors have no relevant financial or non-financial interests to disclose in this work.

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