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Original Article

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Causal associations of obstructive sleep apnea with cardiovascular disease: a Mendelian randomization study

Ye Li[1,2,#], Yuyang Miao[1,2,#] and Qiang Zhang[1,2,*]

[1]Department of Geriatrics, Tianjin Medical University General Hospital, Tianjin, China and
[2]Tianjin Geriatrics Institute, Tianjin, China

#Ye Li and Yuyang Miao contributed equally to this work.
*Corresponding author. Qiang Zhang, Department of Geriatrics, Tianjin Medical University General Hospital, Tianjin Geriatrics Institute, Anshan Road No. 154, Tianjin, 300052, China. Email: zhangqiangyulv@163.com.

Abstract

Study Objectives: Obstructive sleep apnea (OSA) had been associated with various cardiovascular diseases (CVDs) in observational studies, but causal inferences have not been confirmed. We used the Mendelian randomization (MR) study to explore the potential causal association between OSA with CVDs in the general population.

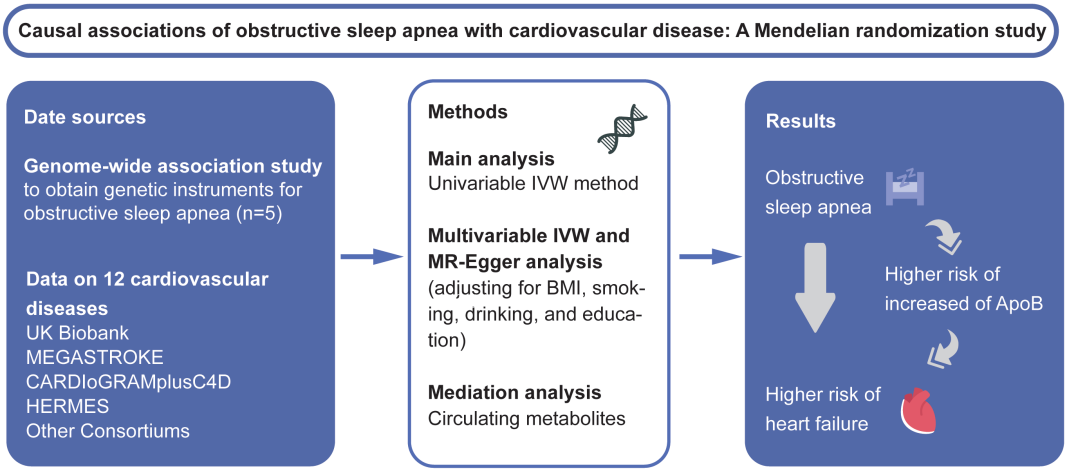
Methods: We performed a two-sample MR analysis using five gene-wide significant single-nucleotide polymorphisms associated with OSA at genome-wide significance from the FinnGen study (N = 217 955) and 12 cardiovascular diseases from the UK Biobank and the genetic consortia. The inverse-variance weight was chosen as the primary analysis and was complemented by various sensitivity analyses. The study design applied univariable MR, multivariable MR, and mediation analysis.

Results: MR analyses provide evidence of genetically predicted OSA on the risk of heart failure (odds ratio [OR],1.26; 95% confidence interval [CI],1.08 to 1.47), hypertension (OR,1.24; 95%CI, 1.11 to 1.39) and atrial fibrillation (OR,1.21; 95%CI,1.12 to 1.31). Multivariable MR indicated the adverse effect of OSA on heart failure persisted after adjusting BMI, smoking, drinking, and education (IVW OR,1.13; 95%CI, 1.01 to 1.27). However, the significance of hypertension and atrial fibrillation was dampened. Mediation analyses suggest that the causal association between OSA and heart failure is mediated in part by Apolipoprotein B, with a mediated portion of 9%.

Conclusions: This study suggested that genetically predicted OSA is a potential causal risk factor for heart failure based on a large-scale population. Nevertheless, further studies regarding ancestral diversity are needed to confirm the causal association between OSA and CVDs.

Key words: Obstructive sleep apnea; Cardiovascular disease; Mendelian randomization; Single-nucleotide polymorphisms

Graphical Abstract



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Statement of Significance

Observational studies have found a causal association between obstructive sleep apnea (OSA) and a wide range of cardiovascular diseases. However, no clear consensus about the effect of sleep apnea on cardiovascular diseases can be drawn as observational studies are susceptible to confounding and reverse causality. We conducted a Mendelian randomization study to determine the association using data from large-scale genome-wide association studies. Our results provide suggestive evidence to support the adverse association between OSA and risk of heart failure, partly mediated by Apolipoprotein B. Given that OSA and cardiovascular disease are health issues with high prevalence worldwide and a huge financial cost, our novel findings have important clinical implications.

Introduction

Obstructive sleep apnea (OSA) is characterized by a sleep-related breathing disorder that affects approximately 34% of men and 17% of women in middle age, including an estimated 24 million undiagnosed persons in the United States [1, 2]. Growing evidence indicates OSA was a major independent risk factor for a wide range of cardiovascular diseases (CVDs), with an underlying pathophysiological of intermittent hypoxia, oxidative stress, and inflammation [3–5]. It is estimated that CVDs account for approximately 31% of all deaths and become a huge economic cost globally, costing about $920 billion in direct medical by 2030 [6]. According to the report, the prevalence of OSA is as high as 40%–80% in patients with stroke, heart failure, coronary artery disease, atrial fibrillation, and hypertension [6]. Although the evidence indicated that people with OSA are likely to be at increased risk of CVDs, it is unclear whether the association is derived from the pathophysiological of OSA or merely the role of confounding factors and reverse causation bias, because most evidence based on observational studies [7–9]. Given some limitations in traditional observational studies, reverse causality occurs when the association is not due to the exposure causing the change in the outcome, but rather the outcome causing the change in the exposure [10]. OSA and CVDs share many risk factors and comorbidities, including obesity, male gender, advanced age, metabolic syndrome, and hypertension, which are often also responsible for confounding bias [11]. Therefore, demonstrating a causal association between OSA and CVDs has been challenging due to shared comorbidities and co-existing states [12]. A better understanding of OSA causally related to specific CVDs would contribute to preventing potential adverse outcomes.

Considering the tendency to expensive and time-consuming nature of randomized controlled trials (RCT), alternative methods of strengthening causal inference may be helpful in directing whether such trials are potentially reasonable. Mendelian randomization (MR) design is an alternative technique for appraising causal inference in epidemiological studies by utilizing genetic variants as instrumental variables [13, 14]. The advantage of MR is that genetic variants are randomly assigned when passed from parents to offspring and irrelevant to self-selective behavior, which can strengthen the causal inference by reducing potential unmeasured residual confounding and avoiding the reverse causality [15]. Although MR designs have recently been used to investigate the association between OSA and the risk of a few CVDs [12, 16], the causality for a wide range of CVDs, such as heart failure, myocardial infarction, and venous thromboembolism, is still unclear. Here, we employed a two-sample MR study to thoroughly disentangle the causal association between OSA and the risk of CVDs.

Methods

Study design

Figure 1 provides a summary of the design of the study. We performed two-sample MR analyses to explore the causal association of OSA with the risk of a wide range of CVDs. MR analysis uses genetic variants as instrumental variables to estimate the causal association between exposure and development of disease [17]. The genetic variants follow three assumptions: (1) reliably and robustly related to the exposure, (2) independent of risk factor-outcome confounders, and (3) influence the outcome only via the exposure [15]. The analysis was conducted with summary-level data from recently publicly available genome-wide association studies (GWASs). Univariable MR was performed to explore the causal relationship between OSA and 12 CVDs, and then multivariable MR was used to further assess the direct effect of OSA on heart failure, hypertension, and atrial fibrillation, independent of possible confounders (e.g. BMI, smoking, drinking, and education). In the end, we used two-step MR to assess whether possible mediators such as circulating metabolites mediate the causal association between OSA and heart failure. All GWAS summary statistics used in present study have been publicly available, and ethical approval was already obtained in the original studies.

Data sources and instrument variables

Obstructive sleep apnea.

We selected five single-nucleotide polymorphisms (SNPs) recently demonstrated to be associated with the OSA traits among 217 955 individuals of European ancestry [18]. The GWAS study involved 16 761 patients with OSA identified through the Finland nationwide health registries. The diagnosis of OSA was according to the International Classification of Diseases (ICD-10: G47.3, ICD-9: 3472A), following self-reported symptoms, clinical examination, and sleep registration applying apnea-hypopnea index (AHI) ≥ 5/hour or respiratory event index (REI) ≥ 5/hr [18]. Two SNPs (rs9937053 and rs4837016) were located in *FTO* and *GAPVD1* are significantly correlated with various biological activities in OSA, which may be the potential target gene variants sites. The rs10507084 were located in *RMST/NEDD1*, rs185932673 near *CAMK1D,* and rs10928560 near *CXCR4*. The details of the SNPs selected as instrumental variables are available in Supplementary **Table S1**.

12 CVDs.

We used summary-level data of 12 CVD outcomes derived from large genetic consortia [19–24], and the Pan UK Biobank. The 12 cardiovascular diseases include coronary artery disease, myocardial infarction, hypertension, atrial fibrillation, heart failure,

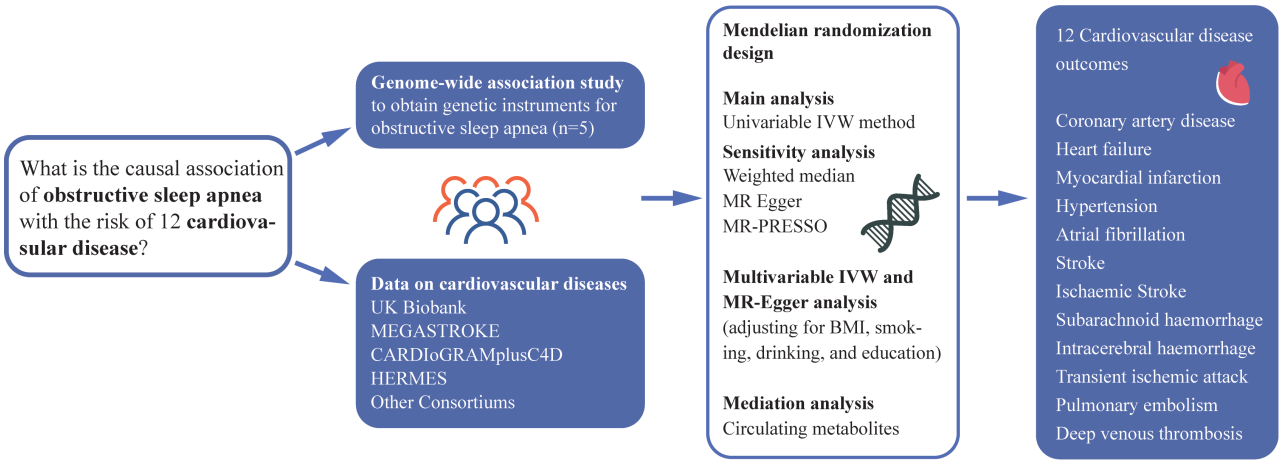

Figure 1. Overview of the design and main results of this Mendelian randomization (MR) study on obstructive sleep apnea risk factors and cardiovascular disease. MR, Mendelian randomization. MR-PRESSO, MR pleiotropy residual sum and outlier test; IVW, inverse-variance weighted; CARDIoGRAMplusC4D, Coronary Artery Disease Genome-wide Replication and Meta-analysis plus The Coronary Artery Disease Genetics; HERMES, Heart Failure Molecular Epidemiology for Therapeutic Targets.

stroke, ischemic stroke, intracerebral hemorrhage, subarachnoid hemorrhage, transient ischemic attack, pulmonary embolism, and deep venous thrombosis. A detailed description of the data sources of the outcomes is displayed in Supplementary **Table S2**.

Potential pleiotropy.

We drew summary statistics data for drinks per week (N = 630 154), smoking initiation (N = 249 171), education (N = 765 283), and BMI (N = 322 154) from the Sequencing Consortium of Alcohol and Nicotine use (GSCAN) [25], the Social Science Genetic Association Consortium (SSGAC) [26] and the Genetic Investigation of Anthropometric Traits (GIANT) [27](Supplementary **Table S2**). We excluded data from UK Biobank in the smoking initiation and drinks per week GWAS summary statistics to avoid population overlap.

Potential mediators.

We used summary statistics of genome-wide association studies associated with potential mediators (circulating metabolites), including: apolipoprotein A-I (ApoA-I), Apolipoprotein B (ApoB), serum total cholesterol, LDL cholesterol, and HDL cholesterol [28]. All metabolites were adjusted for age, sex, and time since the last meal (if applicable), and the final sample size was 24925 [28] (Supplementary **Table S2**).

Selection of the genetic instrumental variables.

Identified SNPs with meaningful at the genome-wide significance level ($p < 5 \times 10^{-8}$). The included SNPs were located in different gene regions and without linkage disequilibrium($r^2 < 0.001$) estimated based on the 1000 Genomes European reference panel [18]. We searched for each included instrumental variable from PhenoScanner to evaluate any previous associations with potential confounders [29, 30]. To meet the assumptions of the MR design, we excluded SNPs that were strongly associated with the outcome ($p < 5 \times 10^{-8}$). The details of SNPs used as instrumental variables and their associations with the outcomes are shown in (Supplementary **Tables S1, S5, and S7**).

Strength of instrumental variables.

To evaluate the weak instrument bias of the instrumental variables, we calculated the F-statistic based on the following equation: $F = R^2/(1\text{-}R^2) * (N\text{-}k\text{-}1)/k$ [31]. The R^2 is the proportion of risk factor variability explained by genotype, N is the SampleSize, and k is the number of IV. The calculation of R^2 using the formula: $R^2 = [beta.exposure^2]/[se.exposure^2 * N + beta.exposure^2]$ [32]. An F-statistic > 10 indicates a low probability of a weak instrument bias [31].

Statistical analysis

Univariable MR.

The multiplicative random-effect inverse-variance weighted (IVW) models as selected as the main MR analysis methods and were performed in each cohort respectively [33]. Fixed-effects meta-analysis method was used to combine the odds ratios(OR) estimates from different sources for one endpoint [34]. The IVW method provides the most accurate estimates although doesn't correct for invalid instrument bias and pleiotropic effect if present [33]. We conducted several sensitivity analyses including weighted median [35], MR-Egger regression [36], and MR-PRESSO [37] to detect the validity and robustness of the founding. The weighted median method for estimating causal effects permits at most 50% of the instrumental variables to breach the MR assumption in the presence of horizontal pleiotropy [35]. The MR-Egger regression was performed to test the directional pleiotropy by its intercept [36]. The MR-PRESSO method is intended to identify and correct for outlying SNPs and check whether causal assessment estimates changed after excluding possible outliers that are potentially horizontal pleiotropy [37]. In IVW analysis, we used Q statistics and I^2 index to assess heterogeneity between instrumental variables in the fixed-effect variance-weighted analysis [35]. If the Q statistic indicated the presence of heterogeneity ($p < 0.05$), then we used a random-effects model IVW analysis to assess causal effects. The effect estimates of genetically predicted OSA on CVDs were presented as ORs with their 95% CIs per 1-unit- higher log odds of OSA. The two-sided P-value below 0.05 was considered suggestive of association. All statistical analyses were conducted in Stata/SE 16.0 and R project version 4.1.3. MR analysis is performed by applying the TwoSampleMR package [38]. A forest plot is generated using the forest plot package.

Multivariable MR.

The multivariable MR is developed as an extension of univariable MR, which allows assessment of the common causal effects of a variety of risk factors on CVD risk by integrating all interested exposures into the single model. Considering the strong genetic association between OSA and BMI and to adjust for potential pleiotropy (that is, smoking, drinking, and education), we applied multivariable MR analysis to estimate the direct effect of OSA on CVDs under consideration of multiple exposure scenarios of interest [18, 39, 40]. We extracted the instrumental variables of BMI, smoking, drinking, and education ($p < 5 \times 10^{-8}$) from the corresponding genome-wide association studies and integrated them with the existing exposure instrumental variables of OSA, then excluded duplicate SNPs and clumped by linkage disequilibrium ($r^2 < 0.001$ estimated based on the 1000 Genomes European reference panel) to ensure that SNPs were independent. Effects and corresponding standard errors for each SNP were acquired from data for the corresponding exposures and outcomes. We applied IVW [41]and MR-Egger [42] to estimate causal effects in multivariable MR analyses. MR analysis is performed by applying the TwoSampleMR and MendelianRandomization package [38].

Mediation analysis.

For significant MR associations, we used a two-step MR to assess the degree to whether the effect of OSA on risk of heart failure is mediated by circulating metabolites or not. In the first step, we estimate the causal association between OSA and circulating metabolites. In the second step, instrumental variables of the identified mediators were used to predict circulating metabolites on heart failure risk. Where there was evidence that OSA affects circulating metabolites, which in turn influenced heart failure, we used the "product of coefficients" method [43] to assess the direct indirect effect of OSA on heart failure risk via each mediator. The standard errors of indirect effects were derived from the use of the delta method [44].

Results

Univariable MR

The associations of OSA predicted by 5 selected SNPs with 12 CVD outcomes are demonstrated in **Figure 2**. Genetically predicted OSA was positively associated with risk of several CVDs, including heart failure, hypertension, and atrial fibrillation. For one-unit increase in log odds of genetically predicted OSA, the OR was 1.26 (95% CI, 1.08 to 1.47; $P = 0.004$) for heart failure, 1.24 (95% CI, 1.11 to 1.39; $P < 0.001$) for hypertension and 1.21(95% CI,1.12 to 1.31; $P < 0.001$) for atrial fibrillation. However, there were no significant associations were found between genetically predicted OSA and risk of other CVDs, such as coronary artery disease, myocardial infarction, any stroke, and venous thromboembolism.

The estimated F-statistic of the instrumental variable we selected for OSA was 41, suggesting a small magnitude of weak instrument bias. The effect estimator remained directionally consistent in the weighted median analysis (Supplementary **Table S3**), and no directional pleiotropy was found in the MR-Egger analysis for most studies (Supplementary **Table S3**). Heterogeneity tests detected that there may be some heterogeneity across the individual SNP effect estimates, and two outliers were identified in MR-PRESSO for myocardial infarction (Supplementary **Table S4**). After removing outliers in MR-PRESSO analysis, the causal effect estimates of OSA were basically consistent with the results of main analysis.

Multivariable MR

Given the possible effects of BMI, smoking, drinking, and education in estimating the causal association, we performed a multivariable MR analysis to infer the direct effect of OSA on CVDs (including heart failure, hypertension, and atrial fibrillation) [39, 45]. Evidence of a direct causal effect of OSA on risk of heart failure (controlling for smoking: IVW OR,1.35; 95% CI, 1.18 to 1.54; $P < 0.001$; controlling for drinking: IVW OR,1.28; 95% CI, 1.12 to 1.48; $p < 0.001$; controlling for BMI: OR,1.18; 95% CI,1.02 to 1.37; $p = 0.031$; controlling for education: IVW OR, 1.16; 95% CI,1.02 to 1.33; $p = 0.023$) (**Table 1**). When we consider all interested exposures simultaneously in the same model, the effect value becomes slightly weaker (IVW OR,1.13; 95% CI, 1.01 to 1.27; $p = 0.034$), whereas the associations between genetically predicted OSA and hypertension (IVW OR,1.05; 95% CI, 0.96 to 1.14; $p = 0.29$) and atrial fibrillation (IVW OR,1.06; 95% CI, 0.97 to 1.15; $p = 0.21$) risk were not statistically significant (**Table 1**). The multivariate MR-Egger estimates are essentially similar to the IVW analysis, although the CIs are wider than IVW, which may be due to the relatively smaller power [46]. Furthermore, the MR-Egger intercept term indicated that effect estimates for OSA are unlikely to be distorted by horizontal pleiotropy in multivariate MR analyses, as *P*-values were all well above 0.05.

Mediation analysis

Given the higher cardiometabolic risk in OSA patients, the measurement of circulating metabolites may be mediator underlying the adverse effect of OSA on heart failure [47]. We examined the mediating pathways of Apo A-I, ApoB, total serum cholesterol, HDL cholesterol, and LDL cholesterol in OSA on risk of heart failure by a two-step MR analysis (**Figure 3**). In the first step, we used the selected OSA instrumental variables to assess the causal relationship with circulating metabolites. Summary information on the five selected OSA-associated SNPs associated with circulating metabolite phenotypes is presented in Supplementary **Table S5**. We assessed a causal relationship between OSA and ApoB among five possible mediators. For a one-unit increase in log odds of genetically predicted OSA, the IVW beta was 1.26 (95% CI, 0.02 to 0.22; $p = 0.019$) for ApoB (**Table 2 and** Supplementary **Table S6**). In a second step, we investigated the causal effect of mediators on heart failure using instrumental variables of circulating metabolites (Supplementary **Table S7**). There was causal evidence suggests that ApoB (IVW OR,1.19; 95% CI, 1.01 to 1.41; $p = 0.035$) and LDL cholesterol (IVW OR,1.17; 95% CI, 1.03 to 1.34; $p = 0.018$) cause an increased risk of heart failure (**Table 2** and Supplementary **Table S8**). Estimation of the weighted median and MR-Egger are consistent with the direction of the IVW MR analysis. Finally, we estimated the causal effect of OSA on heart failure through the mediating effect of ApoB as 0.02(95% CI, 1.00 to 1.05) with a mediated proportion of 9% (Table 3).

Discussion

This MR study based on large-scale genetic consortia investigated the potential causal role of OSA in a wide range of CVDs. We observed evidence indicating an adverse causal effect of OSA on heart failure, hypertension, and atrial fibrillation. However, the significance of hypertension and atrial fibrillation dampened in subsequent multivariable MR analyses. Our results are largely robust based on strong instruments and across different MR analysis methods that make different hypotheses about horizontal pleiotropy. In addition, we performed a mediation analysis for the

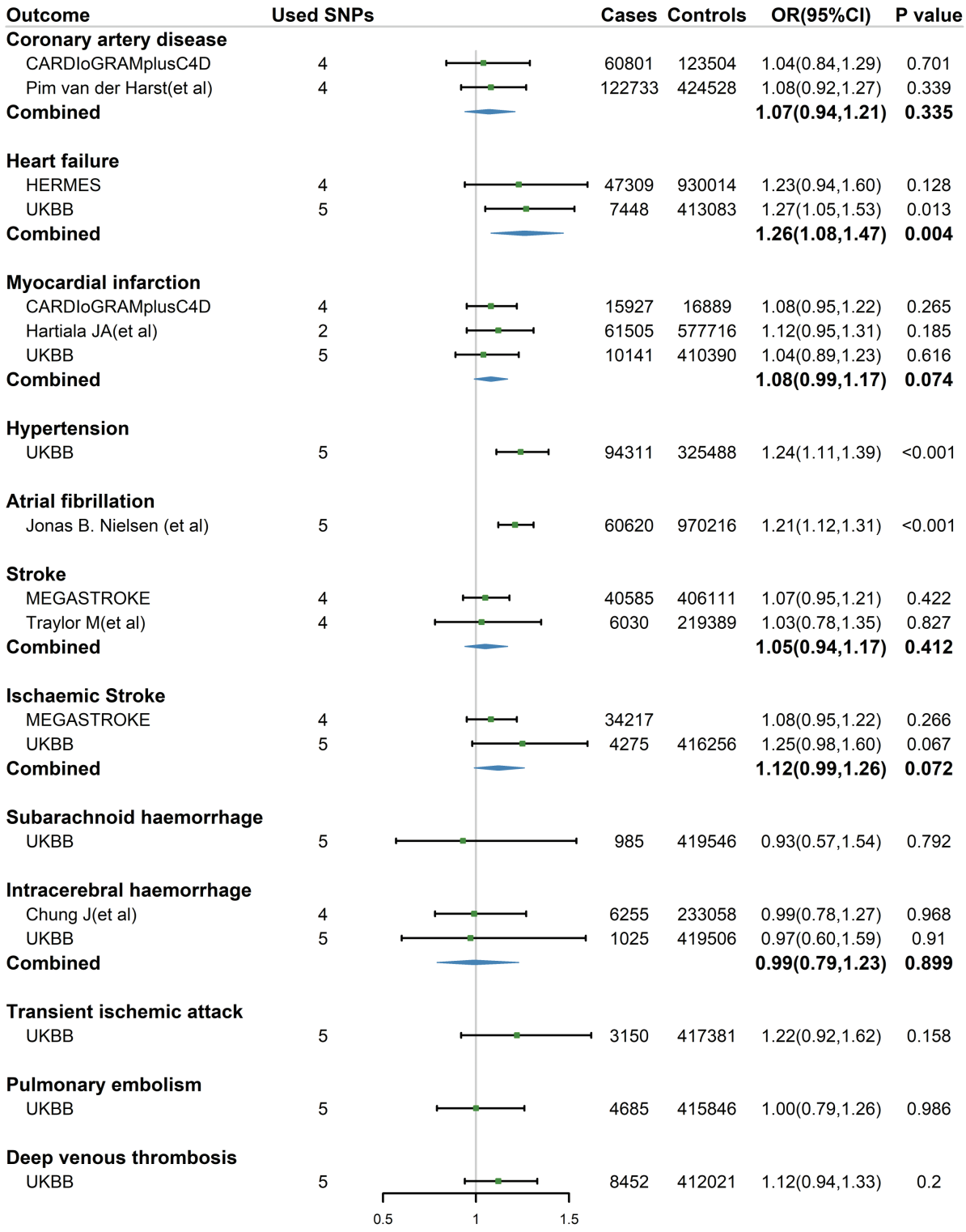

Figure 2. Associations of genetically predicted obstructive sleep apnea with risk of cardiovascular disease. CARDIoGRAMplusC4D, Coronary Artery Disease Genome-wide Replication and Meta-analysis plus The Coronary Artery Disease Genetics; CI, confidence interval; HERMES, Heart Failure Molecular Epidemiology for Therapeutic Targets; OR, odds ratio; SNP, single-nucleotide polymorphism; UKBB, UK Biobank

causal effect of OSA and heart failure and found that ApoB may be played a partial mediator effect.

As far as we know, no previous MR study has investigated the association between genetically predicted OSA with a broad range of cardiovascular and cerebrovascular diseases and venous thrombotic outcomes. The current evidence on association between OSA and CVDs is almost exclusively based on sleep-clinic and cross-sectional studies in patients with multiple comorbidities and a history of CVDs, and the findings may be limited by confounding influences [48–52]. With regard to heart failure, our finding supports the results from a prospective study of 4422 participants with OSA who had an increased risk of heart failure

Table 1. Multivariable MR results of OSA on risk of CVD

Model	Outcome	Number of SNPs	MVMR-IVW	*p*	MVMR-Egger	*p*
			OR(95% CI)		OR(95% CI)	
MVMR1	Heart failure	12	1.35(1.18,1.54)	<0.001	1.34(1.13,1.60)	0.001
	Hypertension	12	1.24(1.02,1.51)	0.028	1.31(0.97,1.76)	0.078
	Atrial fibrillation	12	1.20(1.07,1.34)	0.001	1.18(0.99,1.40)	0.061
MVMR2	Heart failure	11	1.28(1.12,1.48)	<0.001	1.24(1.02,1.53)	0.035
	Hypertension	11	1.51(0.87,2.62)	0.140	1.12(0.87,1.42)	0.383
	Atrial fibrillation	11	1.19(1.10,1.29)	<0.001	0.92(0.55,1.55)	0.759
MVMR3	Heart failure	64	1.18(1.02,1.37)	0.031	1.09(0.91,1.30)	0.339
	Hypertension	64	0.93(0.77,1.12)	0.430	0.97(0.75,1.26)	0.832
	Atrial fibrillation	63	1.01(0.90,1.14)	0.810	0.95(0.81,1.11)	0.484
MVMR4	Heart failure	341	1.16(1.02,1.33)	0.023	1.17(1.07,1.27)	0.001
	Hypertension	341	1.13(1.06,1.20)	<0.001	1.16(1.05,1.27)	0.832
	Atrial fibrillation	342	1.07(1.01,1.14)	0.033	1.13(1.03,1.25)	0.031
MVMR5	Heart failure	179	1.13(1.01,1.27)	0.034	1.10(0.97,1.25)	0.134
	Hypertension	178	1.05(0.96,1.14)	0.290	1.06(0.93,1.21)	0.371
	Atrial fibrillation	179	1.06(0.97,1.15)	0.210	1.08(0.95,1.24)	0.242

MVMR1, multivariable MR analysis adjusting for smoking; MVMR2, multivariable MR analysis adjusting for drinking; MVMR3, multivariable MR analysis adjusting for BMI; MVMR4, multivariable MR analysis adjusting for education; MVMR5, multivariable MR analysis adjusting for smoking, drinking, BMI and education. OSA, Obstructive sleep apnea; SNPs, single-nucleotide polymorphism; MR, Mendelian randomization; OR, odd ratio.

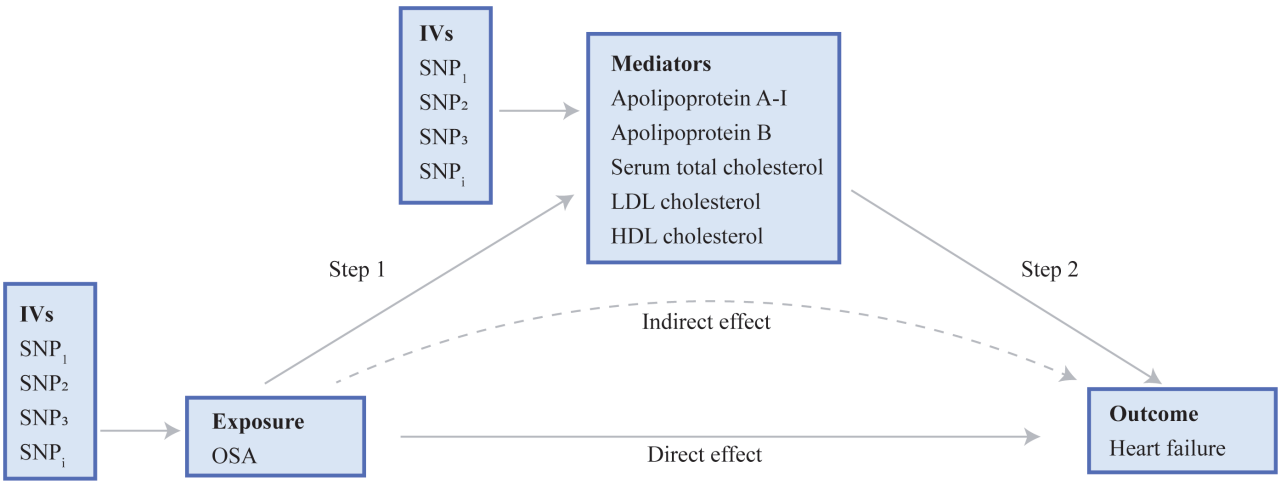

Figure 3. The design of mediation analysis of the effect of Obstructive sleep apnea (OSA) on heart failure via potential mediators. Step 1 evaluated the causal effect of the OSA on the potential mediators, and step 2 assessed the causal effect of the mediators on heart failure risk. "Direct effect" indicates the effect of OSA on heart failure risk after adjusting for the mediator. "Indirect effect" indicates the effect of OSA on heart failure risk through the mediator. IVs, instrumental variables.

(OR,1.3; 95% CI,1.02 to 1.26) with a mean follow-up of 8.7 years [3]. A RCT study showed significant improvement in cardiac function and hypoxemia in patients with OSA with heart failure with automatic positive airway pressure intervention [53]. Early identification and hospitalization of OSA in patients with heart failure is feasible and resulted in improvement in systolic function, as demonstrated in other RCT studies [54, 55]. Although another RCT study did not observe an association between OSA and cardiovascular events, this may be due to the fact that the diagnosis of OSA was not well established at the time and the CPAP time was less than 4h/night [51]. Meanwhile, a recently published MR study proposed a suggestive association between sleep apnea and coronary heart disease, while no significant association was found with heart failure (*N* = 977 323) [12]. Our study provided suggestive evidence of a direct causal association between OSA and heart failure based on a larger population, and the association remained significant after adjusted potential pleiotropy in multivariable MR model. To date, 6 completed studies are testing the abovementioned association (Supplementary **Table S9**) [56]. In effect, in contrast to the short-term influence seized in traditional observational studies, the MR design estimates reflect a lifelong sleep apnea exposure and are independent of confounding and reverse causation.

Although there have been quite a few studies on the association between OSA and atrial fibrillation or hypertension, the findings had been inconsistent and inconclusive [57–60]. A recently

Table 2. The mediation analysis results of OSA on heart failure via circulating metabolites

	Number of SNPs	Beta(95% CI)	*p*
OSA on circulating metabolites			
Apolipoprotein A-I	5	0.02(−0.08,0.12)	0.667
Apolipoprotein B	5	0.12(0.02,0.22)	0.019
Serum total cholesterol	5	0.10(0.00,0.20)	0.056
LDL cholesterol	5	0.08(−0.04,0.19)	0.198
HDL cholesterol	5	−0.07(−0.16,0.03)	0.152
Circulating metabolites on heart failure			
Apolipoprotein A-I	8	0.08(−0.07,0.22)	0.305
Apolipoprotein B	7	0.18(0.01,0.36)	0.035
Serum total cholesterol	13	0.10(−0.05,0.25)	0.192
LDL cholesterol	14	0.16(0.03,0.29)	0.018
HDL cholesterol	8	0.07(−0.09,0.23)	0.400

Summary MR estimates derived from the inverse-variance weighted (IVW) methods for the effect of Obstructive sleep apnea (OSA) on circulating metabolites and the effect of circulating metabolites on heart failure. The error bars represent 95% CIs. All statistical tests were two-sided. $p < 0.05$ was considered significant. SNPs, single-nucleotide polymorphism.

Table 3. The mediation effect of OSA on heart failure via Apolipoprotein B

Mediator	Total effect	Direct effect A	Direct effect B	Mediation effect	Mediated proportion(%)
	Beta(95%CI)	Beta(95%CI)	Beta(95%CI)	Beta(95%CI)	
Apolipoprotein B	0.24(0.05,0.42)	0.12(0.02,0.22)	0.18(0.01,0.36)	0.02(1.00,1.05)	9

Total effect' indicates the effect of OSA on Apolipoprotein B, 'direct effect A' indicates the effect of OSA on Apolipoprotein B, 'direct effect B' indicates the effect of Apolipoprotein B on heart failure, and 'mediation effect' indicates the effect of OSA on heart failure through Apolipoprotein B. Total effect, direct effect A and direct effect B were derived by inverse-variance weighted (IVW); mediation effect was derived by using the delta method.

published MR study suggested a significant causal association between OSA and atrial fibrillation [16]. While this causal association was not significant after multivariable MR adjustment for BMI in present study, implying that BMI may play a major role in atrial fibrillation risk rather than OSA. Notably, the effect of CPAP on lowering blood pressure and ameliorating atrial fibrillation in patients with OSA had been discouraging and inconsistent. A meta-analysis that included 31 RCT studies demonstrated reductions of 2-3 mmHg of blood pressure after CPAP treatment in patients with OSA [61]. There was insufficient evidence to support a direct causal association between OSA and heart failure or hypertension after multivariable MR analysis in this study. Indeed, OSA and multiple CVDs are common conditions caused by a variety of factors and often coexist, many clinically observed CVDs are not necessarily a consequence of OSA.

The implication of setting up multivariate MR analysis in this study is to evaluate the causal effect of multiple OSA-related exposures on the outcome, with the advantage that the direct effect of OSA on CVDs can be robustly estimated in different scenarios, thus avoiding the confounding, mediating, or pleiotropic pathway effects exerted by secondary exposures [40]. More convincingly, increasing genetic liability to OSA was associated with heart failure risk, after adjusting smoking, drinking, BMI, and education respectively, and in a model considering all interested exposures simultaneously.

Considering genetically predicted OSA associated with an increased risk of heart failure, we subsequently performed a two-step MR for mediation analysis and identified that part of effect was may mediated by ApoB. A recent genome-wide association study on sleep and lipid profiles confirmed that ApoB follows circadian rhythms in multiple metabolic organs, thus establishing that sleep disruption may affect lipid biological functions [62]. Besides, a clinical cohort study found that rapid eye movement AHI was independently associated with ApoB in patients with OSA [63], and that good CPAP compliance reduced ApoB levels were confirmed in several clinical trials [64, 65]. The above evidence is supported by our first step MR analysis. Six studies were identified where the impact of OSA on ApoB had been assessed (Supplementary **Table S10**). The second step MR analysis delivered evidence that genetically determined high levels of ApoB and LDL cholesterol and increased risk of heart failure. The ApoB to Apo A-I ratio was determined strongly associated with heart failure per gender in a large prospective study in Sweden that included 84 740 participants over 11.8 years [66], and the ApoB/ApoA-1 ratio was the strongest predictor of heart failure also confirmed in RCT study [67]. Summary characteristics of the studies are presented in Supplementary **Table S11**. Meanwhile, the circulating lipidomic network screening indicated a significant association between lipid scores and risk of heart failure [68]. Overall, the above evidence demonstrates a possible causal association between ApoB and heart failure.

From a pathophysiological mechanistic perspective, OSA is likely to increase the risk of CVDs through several pathways, especially given the shared comorbidities. OSA is characterized by chronic systemic inflammation, inducing oxidative stress, and increasing sympathetic nerve activation [69]. During the sleep apnea event, excessive negative intrathoracic pressure leads to overload and destruction of ventricular function, which contributes to diastolic dysfunction [70]. Additionally, activation of the sympathetic nervous system causes abnormal fluctuations in

heart rate and blood pressure [71]. The increase in oxidative stress leads to the overexpression of adhesion molecules and cytotoxicity of monocytes, and promotes the progression of vascular dysfunction [72]. The disruption of vascular homeostasis will lead to a series of pathological events such as excessive vasoconstriction, thrombosis, hypercoagulability, and increased inflammatory factors [73, 74]. Furthermore, the impact of ApoB-containing particles on the risk of atherosclerotic cardiovascular disease seems to be driven primarily by the concentration of circulating ApoB particles, rather than by the mass of cholesterol carried by these particles (LDL-c) [75]. Therefore, future treatments aimed at lowering triglyceride or cholesterol should be based on reducing the absolute amount of ApoB levels, and reversing early signs of atherosclerosis before major cardiovascular events occur is critical for patients with OSA.

This study following the MR guidelines elucidated the causal association between genetic liability for OSA and a wide range of CVDs with several strengths [76]. The core advantage is that MR design, which strengthens the causal inference by removing residual confounding and reverse causation in the observational studies. In addition, we limited the population to European to minimize the bias due to ethnic heterogeneity. Another strength is that we estimated the causality of genetically predicted OSA with CVDs utilizing several data derived from different sources, which ensured the reality and robustness of the results and largely reduced population stratification.

Undeniably, the results of this study should be carefully interpreted in the context of its limitations and those of MR in general. First, although we selected instrumental variables strongly associated with OSA, genetic variation represents a small fraction of the disease and cannot be considered an accurate proxy for exposure. Second, the overlap of approximately 50% of the dataset between education and CVD from the UK Biobank may bias the assessment toward observing correlations in the presence of weak instruments. Considering that all selected instrumental variables are strongly correlated with education, weak instrument bias should be minimal [77]. Third, considering OSA is binary exposure, the instrumental variable estimate would be the average causal estimate in those individuals whose OSA is influenced by the presence or absence of the genetic variants used [78]. Applying traditional MR analysis to binary exposures may produce unidentifiable relative risk values in which boundaries can be identified, but not exact causal effects. Therefore, we conceptualize dichotomous exposures as a coarse approximation of the underlying continuous risk factor. Fourth, since we cannot fully specify the biological role of genetic instruments currently, the possibility of violating the independence and exclusion restriction assumptions, especially with respect to pleiotropy, cannot be completely ruled out. However, we used several methods to verify the robustness of causal estimates, including sensitivity analysis using Cochran's Q statistic, MR-PRESSO, weighted median, and MR-Egger. Fifth, there may be instances of misclassification by their own confounding due to genetic variants being proxies for causal variants [79], which affect the identifiability and interpretation of the effect of a difference in the distributions of the risk factor between genetically determined subgroups [80]. While the misclassification may not affect the validity of the test, but the statistical validity of the test is relatively low [79]. Sixth, the presence of weak instrument bias could not be assessed due to the partial overlap of the exposure datasets in the multivariable MR analysis, which requires individual-level data to estimate the conditional F statistic. Seventh, the results of the MR analysis reflect the effects of lifelong sleep apnea exposure, which may produce stronger effects than limited-time observational studies. Therefore, RCT experiments are also needed to further confirm the causal relationship between OSA and CVDs. Finally, our results are mainly based on European ancestry, which restricted the generalization of the results to other populations.

Conclusions

This MR study supports the hypothesis that OSA may be implicated in the development of CVDs. We found suggestive evidence for adverse association between OSA and risk of heart failure, partly mediated by ApoB, which delivers that further strategies need to be explored for the detection and treatment of OSA to mitigate the potential adverse cardiovascular events. Nevertheless, further studies are needed to confirm the association between OSA and other CVDs.

Supplementary Material

Supplementary material is available at *SLEEP* online.

Table S1-S11

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Disclosure Statement

Financial disclosure: None. Nonfinancial disclosure: None.

Competing Interests

The authors declare that they have no competing interests.

Declarations

All studies included in cited genome-wide association studies had been approved by a relevant review board, and participants had provided written informed consent.

Data Availability

Information on how to obtain summary-level data from the FinnGen consortium, the UK Biobank, MEGASTROKE, HERMES, and the CARDIoGRAMplusC4D consortium is available at https://finngen.gitbook.io/documentation/, https://www.nealelab.is/uk-biobank, http://megastroke.org/, https://www.hermesconsortium.org/, and http://www.cardiogramplusc4d.org/data-downloads/, respectively.

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