ESC European Society of Cardiology

European Heart Journal (2021) **42**, 3349–3357
doi:10.1093/eurheartj/ehab170

CLINICAL RESEARCH
Epidemiology and prevention

Causal associations of short and long sleep durations with 12 cardiovascular diseases: linear and nonlinear Mendelian randomization analyses in UK Biobank

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Received 4 November 2020; revised 15 December 2020; editorial decision 5 March 2021; accepted 6 March 2021; online publish-ahead-of-print 5 April 2021

See page 3358 for the editorial comment on this article (doi:10.1093/eurheartj/ehab248)

Aims Observational studies have suggested strong associations between sleep duration and many cardiovascular diseases (CVDs), but causal inferences have not been confirmed. We aimed to determine the causal associations between genetically predicted sleep duration and 12 CVDs using both linear and nonlinear Mendelian randomization (MR) designs.

Methods and results Genetic variants associated with continuous, short (≤6 h) and long (≥9 h) sleep durations were used to examine the causal associations with 12 CVDs among 404 044 UK Biobank participants of White British ancestry. Linear MR analyses showed that genetically predicted sleep duration was negatively associated with arterial hypertension, atrial fibrillation, pulmonary embolism, and chronic ischaemic heart disease after correcting for multiple tests ($P < 0.001$). Nonlinear MR analyses demonstrated nonlinearity (L-shaped associations) between genetically predicted sleep duration and four CVDs, including arterial hypertension, chronic ischaemic heart disease, coronary artery disease, and myocardial infarction. Complementary analyses provided confirmative evidence of the adverse effects of genetically predicted short sleep duration on the risks of 5 out of the 12 CVDs, including arterial hypertension, pulmonary embolism, coronary artery disease, myocardial infarction, and chronic ischaemic heart disease ($P < 0.001$), and suggestive evidence for atrial fibrillation ($P < 0.05$). However, genetically predicted long sleep duration was not associated with any CVD.

Conclusion This study suggests that genetically predicted short sleep duration is a potential causal risk factor of several CVDs, while genetically predicted long sleep duration is unlikely to be a causal risk factor for most CVDs.

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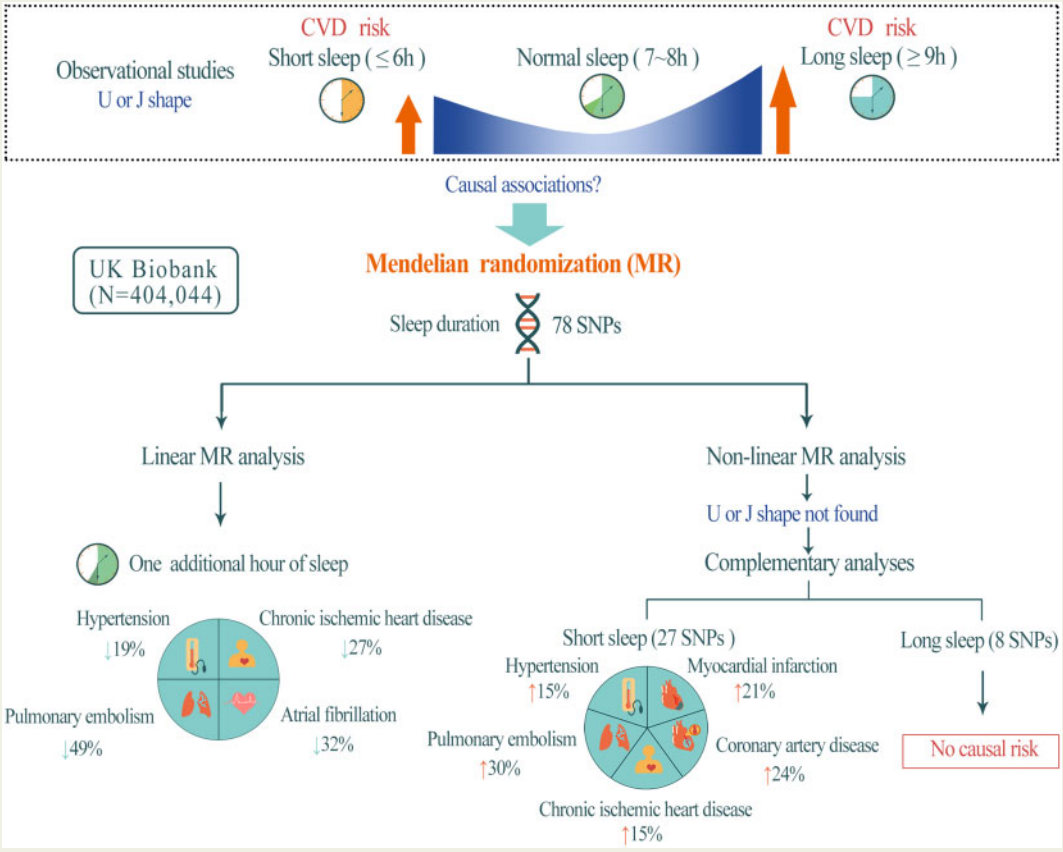
Graphical Abstract



Causal associations of short and long sleep durations with 12 cardiovascular diseases: linear and nonlinear Mendelian randomization analyses in UK Biobank. All associations are significant at a Bonferroni threshold of P < 4.2E-03 (corrected for 12 outcomes). CVD, cardiovascular disease; SNP, single nucleotide polymorphism.

Keywords Short sleep duration • Long sleep duration • Cardiovascular disease • Mendelian randomization • Linear • Nonlinear

Introduction

As a modifiable lifestyle behaviour, sleep is vital to human health. Observational studies have consistently suggested associations between sleep duration and adverse health outcomes.[1–5] Several meta-analyses have shown either U- or J-shaped associations between sleep duration and various cardiovascular diseases (CVDs), indicating that both short (≤6 h) and long (≥9 h) sleep durations are associated with higher risks of CVDs.[6–9] These studies have also shown that these adverse effects are more pronounced in people with longer sleep duration. The associations of short sleep duration with CVDs are supported by experimental studies, which have consistently shown that sleep deprivation or restriction would exert various adverse effects on the cardiovascular systems of healthy participants, including increased activity in the sympathetic nervous system, inflammation, and accelerated atherosclerosis.[10–12] In contrast, no experimental study to date has suggested that sleeping longer than 9 h is inherently harmful, and there is a lack of hypothetical mechanisms in explaining the adverse effects of long sleep duration on CVD risks, especially in healthy participants.[13,14] Although the underlying reasons for long sleep duration leading to increased CVD risks are not highly apparent, many experts have suggested that a long sleep duration is a fatal lifestyle factor in the general population.[9,15,16] However, this statement is potentially an overclaim or even misleading, if the causal role of long sleep duration on cardiovascular outcomes is not clarified.

Traditional observational studies, even when they are well designed, prospective with large sample sizes, are prone to be biased by residual confounding effects and reverse causation. Previous studies have shown that demographic or clinical factors, such as lack of employment, low socioeconomic status, and depression,[17,18] may confound the associations between sleep duration and CVDs.[5]

Mendelian randomization (MR) using genetic variants as instrumental variables can provide unconfounded estimates and overcome the limitations of observational studies. The rationale for the MR design is that the genetic variants are fixed at conception and randomly assigned to individuals. Thus, the design can be conceptualized as a natural experiment and is immune to the effects of residual confounding and reverse causation. Recent studies have employed the linear MR method to investigate the causal associations between sleep duration and several CVDs,[19–23] but the causality for a broader range of CVDs, such as atrial fibrillation, pulmonary embolism, and deep vein thrombosis, is still unclear. Notably, the characteristics of the causal associations between sleep duration and many CVDs are likely nonlinear, as reflected by the U- or J-shaped associations as identified in previous observational studies. To our knowledge, there is no study that has attempted to clarify the potential nonlinear MR associations between sleep duration and CVDs.

In the present study, we aimed to determine the potential causal associations between sleep duration and a broad range of CVDs via MR analyses. We first performed linear MR analyses to estimate the associations between genetically predicted sleep duration and CVD risks. Second, we employed nonlinear MR analyses to characterize the shape of associations between genetically predicted sleep duration and CVDs. Finally, we used genetic variants associated with short and long sleep durations to determine how genetically predicted short and long sleep durations are causally associated with CVDs, respectively.

Methods

Study participants

The UK Biobank (UKB) cohort recruited >500 000 participants aged 40–69 years in 22 assessment centres across the UK between 2006 and 2010. All participants gave informed consent to participate in the study and were required to complete a series of baseline measurements. All the information, such as sleep duration, disease conditions, confounding factors, and genetic variants, is available in the UKB.[24] To minimize the potential confounding caused by ancestry, our main analysis only included unrelated participants of European ancestry. After detailed quality control of the UKB participants and genetic variants, a total of 404 044 participants were included in the analyses. Detailed information regarding participant selection is available in Supplementary material online, *Text S1*.

Ascertainment of exposure and outcomes

The main exposure of the present study was self-reported sleep duration, which was assessed by a standardized question: 'About how many hours of sleep do you get in every 24 hours? (please include naps)' with responses in integer hours. We excluded participants with missing sleep duration data or sleep duration out of 4–11 h/day range to minimize the incredible sleep duration outliers. Continuous sleep duration was classified into short (≤6 h), normal (7–8 h), and long (≥9 h) sleep durations according to previous study.[25]

To characterize the causal associations between genetically predicted sleep duration and different cardiovascular risks, we included a broad range of CVDs, including cerebrovascular diseases (ischaemic stroke, haemorrhagic stroke, and transient ischaemic attack), thromboembolic diseases (pulmonary embolism, deep vein thrombosis), and other CVDs (arterial hypertension, atrial fibrillation, chronic ischaemic heart disease, coronary artery disease, myocardial infarction, cardiomyopathy, and peripheral artery disease). All CVD events were obtained according to the first occurrence of a set of diagnostic codes for those CVD outcomes defined by the 3-digit code of the International Classification of Diseases 10th Revision (https://www.who.int/classifications/icd/en/). The source of first occurrence data was derived from hospital inpatient records, death register, primary care records, and self-report health conditions from the UKB assessment clinics. The definition of each CVD is presented in Supplementary material online, *Table S1*.

Single-nucleotide polymorphisms and genetic risk score as instrumental variables

We used 78 single-nucleotide polymorphisms (SNPs) that were associated with continuous sleep duration at a genome-wide significance threshold ($P < 5 \times 10^{-8}$), as reported in a recent genome-wide association study (GWAS) in the UKB,[25] as genetic instruments (Supplementary material online, *Table S2*). The unweighted genetic risk score (GRS) for each participant was calculated by summing the number of sleep duration-increasing alleles. In general, the unweighted GRS explained 0.61% of the variance in sleep duration (R^2 = 0.61%, *F*-statistic = 2459). In addition, we used the SNPs for short (≤6 h/day) and long (≥9 h/day) sleep durations from the same GWAS in the UKB[25] as instrumental variables in the complementary analyses (Supplementary material online, *Tables S3 and S4*).

Study design

We first used a simple method to investigate the association between unweighted GRS of self-reported sleep duration and 12 CVDs (Supplementary material online, *Text S2*). Then, we used standard linear and nonlinear MR analyses to estimate the causal associations between genetically predicted continuous sleep duration and 12 CVDs. We first performed a linear MR analysis to assess the associations between genetically predicted sleep duration and CVDs. The estimate yielded by the linear MR analysis indicates the average change in the outcome resulting from 1 h increase in sleep duration. Then, we performed a nonlinear MR analysis to estimate the shape of associations between genetically predicted sleep duration and CVDs. Given the nonlinear associations between continuous sleep duration and CVDs, we also used SNPs associated with short and long sleep durations to estimate the causal effects of genetically predicted short and long sleep durations on CVDs in the complementary analyses. The causal estimates [i.e. the odds ratios (ORs) for CVDs] were rescaled so that they could be interpreted for per doubling of genetic liability for short and long sleep durations, as previously described.[26]

Linear Mendelian randomization analyses

We used a two-stage least squares method to assess the associations between genetically predicted sleep duration and 12 CVD outcomes produced by the linear MR analyses. We first regressed the exposures on the GRS and then regressed the outcome on the fitted values of the exposure from the first-stage regression. Both stages were adjusted for age, sex, assessment centres, top 10 principal components of ancestry, and genotyping arrays of the participants. To avoid the potential violation of MR assumptions, we further assessed the validity of the genetic variants by testing the associations of potential confounders with the GRS and repeated our MR analyses with an adjustment included for the confounders. Other MR methods (inverse-variance weighted, weighted median and MR-Egger) were also conducted with 'TwoSampleMR' package to account for potential pleiotropy (Supplementary material online, *Text S3*).[27,28] We also conducted RadialMR[29] analyses using modified second-order weights to identify outliers. We used an α level of 0.05 divided by the number of SNPs being used in the MR analyses. Once the

outliers were identified, the outliers were removed, and the results were reanalysed. We used an online tool (https://sb452.shinyapps.io/power/) to estimate the statistical power of a linear MR analysis (Supplementary material online, *Table S5*).

Nonlinear Mendelian randomization analyses

We used nonlinear MR with a piecewise linear method to assess the nonlinear associations between genetically predicted sleep durations and 12 CVDs.[30] In brief, we divided our sample into three strata according to the residual variation of the continuous sleep duration after regressing on the GRS. Then, we calculated piecewise linear MR estimates in each stratum, and these are referred to as localized average causal effects (LACE) in these strata. We reported the *P*-values from two tests for nonlinearity, the quadratic test and the Cochran's *Q* test.[30]

Complementary analyses

In the complementary analyses, we used the sum of the short or long sleep duration risk alleles multiplied by the GWAS effect sizes and then regressed against these CVDs, adjusting for age, sex, assessment centre, top 10 principal components of ancestry, and genotyping array.[20] The effects estimates were scaled as described in the preceding text. For the sensitivity analysis, we used the weighted median, MR-Egger, and RadialMR methods[27–29] to account for any potential pleiotropy and outliers (Supplementary material online, *Text S3*). The outliers, if any, were removed before data analyses were conducted. To account for multiple testing, we used a Bonferroni-corrected threshold of $P < 4.2 \times 10^{-3}$ ($\alpha = 0.05/12$ outcomes) in our primary analyses. We considered *P*-values between 4.2×10^{-3} and 0.05 as suggestive evidence of associations in the main analyses. All statistical analyses were conducted with R software (version 4.0.0 with packages, R Foundation for Statistical Computing, Vienna, Austria), and the *P*-values obtained are two tailed.

Results

Baseline characteristics

The baseline characteristics are shown in *Table 1*. A total of 404 044 participants [mean age (SD): 56.23 years (8.10), 45.2% male] were included in the final analyses. Participants who slept with normal sleep duration (7–8 h per day) were more likely to be younger, educated and employed; had a lower body mass index and Townsend deprivation index; and had a relatively lower prevalence of most CVDs than those who slept shorter than 6 h or longer than 9 h. They were less likely to be current smokers or have low incomes.

Table 1 Baseline characteristics of participants in the UK Biobank (*n* = 404 044)

	Habitual sleep duration (h)						
	4	5	6	7 or 8	9	10	11
Demographics							
Participants, *n*	3357	16 771	75 802	277 818	24 168	5600	528
Age, years	57.2 ± 7.59	57.3 ± 7.71	56.7 ± 7.77	56.7 ± 8.07	59.1 ± 7.79	59.1 ± 7.80	57.4 ± 8.24
Male sex	1427 (42.5)	7293 (43.5)	36120 (47.7)	127718 (46.0)	10543 (43.6)	2537 (45.3)	224 (42.4)
University or college degree	455 (13.6)	3308 (19.7)	21516 (28.4)	92379 (33.3)	5921 (24.5)	1019 (18.2)	98 (18.6)
Current smoker	624 (18.6)	2388 (14.2)	8746 (11.5)	25328 (9.1)	2495 (10.3)	804 (14.4)	79 (15.0)
Current employed	1349 (40.2)	8818 (52.6)	46679 (61.5)	162128 (58.4)	8492 (35.1)	1388 (24.8)	108 (20.5)
Lower income[a]	1265 (37.7)	4631 (27.6)	14873 (19.6)	47344 (17.0)	6638 (27.5)	2123 (37.9)	221 (41.9)
Townsend deprivation index[b]	-0.03 ± 3.53	-0.82 ± 3.27	-1.34 ± 3.03	-1.72 ± 2.83	-1.54 ± 2.95	-0.81 ± 3.24	-0.65 ± 3.41
Body mass index, kg/m^2	29.0 ± 5.90	28.4 ± 5.29	27.8 ± 4.93	27.1 ± 4.56	27.8 ± 4.89	28.9 ± 5.56	29.0 ± 5.90
Outcomes							
Ischaemic stroke	75 (2.2)	259 (1.5)	865 (1.1)	2999 (1.1)	436 (1.8)	137 (2.4)	12 (2.3)
Transient ischaemic attack	80 (2.4)	355 (2.1)	1259 (1.7)	4407 (1.6)	488 (2.0)	130 (2.3)	21 (4.0)
Haemorrhagic stroke	27 (0.8)	114 (0.7)	439 (0.6)	1559 (0.6)	184 (0.8)	45 (0.8)	2 (0.4)
Pulmonary embolism	87 (2.6)	391 (2.3)	1334 (1.8)	4465 (1.6)	590 (2.4)	178 (3.2)	14 (2.7)
Deep vein thrombosis	108 (3.2)	441 (2.6)	1986 (2.6)	7028 (2.5)	686 (2.8)	183 (3.3)	13 (2.5)
Arterial hypertension	1279 (38.1)	5391 (32.1)	20003 (26.4)	64392 (23.2)	7330 (30.3)	2143 (38.3)	214 (40.5)
Atrial fibrillation	240 (7.1)	1030 (6.1)	3836 (5.1)	13340 (4.8)	1666 (6.9)	480 (8.6)	33 (6.3)
Chronic ischaemic heart disease	472 (14.1)	1728 (10.3)	5984 (7.9)	18862 (6.8)	2485 (10.3)	841 (15.0)	75 (14.2)
Coronary artery disease	299 (8.9)	1030 (6.1)	3538 (4.7)	10727 (3.9)	1496 (6.2)	517 (9.2)	48 (9.1)
Cardiomyopathy	26 (0.8)	113 (0.7)	345 (0.5)	1165 (0.4)	163 (0.7)	59 (1.1)	7 (1.3)
Myocardial infarction	291 (8.7)	992 (5.9)	3356 (4.4)	10232 (3.7)	1422 (5.9)	502 (9.0)	50 (9.5)
Peripheral artery disease	90 (2.7)	387 (2.3)	1645 (2.2)	5796 (2.1)	675 (2.8)	195 (3.5)	12 (2.3)

Values are expressed as mean ± standard deviation, or *n* (%).
[a]Lower income was defined as average total household income before tax <18 000.
[b]Townsend deprivation index was calculated based on the preceding national census output areas prior to participant joining the UK Biobank. Each participant is assigned a score corresponding to their postcode location; a lower score represents lower deprivation. All baseline covariates were associated with sleep duration, with $P < 0.001$ for trend across different stratification.

Association between unweighted genetic risk score of self-reported sleep duration and 12 cardiovascular diseases

The baseline characteristics of participants in different GRS quartiles of self-reported sleep duration are shown in Supplementary material online, *Table S6*. The mean sleep duration was significantly associated with the GRS groups, with $P < 2.0E-16$ for trend across categories. We observed significant associations for the prevalence of pulmonary embolism, arterial hypertension, atrial fibrillation, and chronic ischaemic heart disease, with the GRS quartiles (all *P*-values < 4.2E-03, Supplementary material online, *Table S7*). To further test whether a higher GRS was associated with a lower OR of those CVDs, we investigated the associations between GRS of each category and 12 CVDs, with lowest GRS as the reference group (*Table 2*). Compared to the lowest quartile of GRS, the highest quartile of GRS was associated with a 14.0% lower odds for pulmonary embolism ($P = 3.61E-5$), 4.0% lower odds for hypertension ($P = 2.45E-4$), 7.0% lower odds for atrial fibrillation ($P = 5.82E-4$), and 7.0% lower odds for chronic ischaemic heart disease ($P = 1.52E-4$).

Linear Mendelian randomization analyses of sleep duration with 12 cardiovascular diseases

The linear MR analyses showed overall negative associations between genetically predicted sleep duration and 4 of the 12 CVD outcomes (*Figure 1*), including arterial hypertension, atrial fibrillation, pulmonary embolism, and chronic ischaemic heart disease. The OR per genetically predicted 1-h increase in sleep duration ranged from 0.51 [95% confidence interval (CI) 0.38–0.69; $P = 1.02E-05$] for pulmonary embolism to 0.81 (95% CI 0.74–0.88; $P = 4.54E-06$) for arterial hypertension (*Figure 1*), which were consistent with findings regarding the associations between unweighted GRS of self-reported sleep duration and CVDs (*Table 2*). There was suggestive evidence of negative associations between genetically predicted sleep duration and cardiomyopathy, coronary artery disease, and deep vein thrombosis ($P < 0.05$), whereas no significant associations were found between genetically predicted sleep duration and other CVDs, such as ischaemic stroke, transient ischaemic attack, haemorrhagic stroke, myocardial infarction, and peripheral artery disease.

In the sensitivity analyses, we adjusted for the potential confounders that were significantly associated with the GRS (Supplementary material online, *Table S8*), and the results were still consistent with those of the main analysis (Supplementary material online, *Table S9*). The radial MR analyses indicated outlying genetic variants in some of the outcomes, including one outlier SNP each for atrial fibrillation, coronary artery disease, ischaemic stroke, and transient ischaemic attack, respectively, and six outlier SNPs for arterial hypertension (Supplementary material online, *Figure S1*). The pleiotropic effects of these outliers were shown in the phenome-wide association study analyses of the outlying SNPs with all existing traits in the MR-Base database (Supplementary material online, *Figure S2*). After excluding these outliers, the OR of per genetically predicted 1-h increase in sleep duration for arterial hypertension (OR 0.80; 95% CI 0.70–0.91, $P = 0.001$), atrial fibrillation (OR 0.72; 95% CI 0.58–0.88, $P = 0.002$), coronary artery disease (OR 0.77; 95% CI 0.62–0.95, $P = 0.016$), ischaemic stroke (OR 0.69; 95% CI 0.47–1.00, $P = 0.050$), and transient ischaemic attack (OR 0.91; 95% CI 0.65–1.29, $P = 0.61$) did not

Table 2 Associations between genetic risk score quartiles and 12 cardiovascular diseases in UK Biobank ($n = 404\,044$)

	Genetic risk score					
	Lowest GRS	Intermediate GRS		Highest GRS		*P* for trend	
		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Cerebrovascular diseases							
Ischaemic stroke	Ref	0.96 (0.89–1.03)	0.232	0.95 (0.87–1.03)	0.219	0.97 (0.93–1.02)	0.211
Transient ischaemic attack	Ref	1.00 (0.94–1.06)	0.962	0.97 (0.90–1.04)	0.331	0.98 (0.95–1.02)	0.343
Haemorrhagic stroke	Ref	0.94 (0.86–1.04)	0.262	1.06 (0.94–1.19)	0.357	1.03 (0.97–1.09)	0.376
Thrombotic diseases							
Pulmonary embolism	Ref	0.91 (0.86–0.96)	8.69E-4	0.86 (0.81–0.93)	3.61E-5	0.93 (0.90–0.96)	2.72E-5
Deep vein thrombosis	Ref	0.93 (0.89–0.98)	0.004	0.94 (0.89–0.99)	0.025	0.97 (0.94–0.99)	0.020
Other CVDs							
Arterial hypertension	Ref	0.98 (0.96–1.00)	0.025	0.96 (0.92–0.98)	2.45E-4	0.98 (0.97–0.99)	2.41E-4
Atrial fibrillation	Ref	0.95 (0.91–0.98)	0.002	0.93 (0.89–0.97)	5.82E-4	0.96 (0.94–0.98)	4.75E-4
Chronic ischaemic heart disease	Ref	0.97 (0.95–1.00)	0.082	0.93 (0.90–0.97)	1.52E-4	0.97 (0.95–0.98)	1.65E-4
Coronary artery disease	Ref	0.96 (0.92–0.99)	0.025	0.96 (0.92–1.01)	0.108	0.98 (0.96–1.00)	0.096
Cardiomyopathy	Ref	0.97 (0.87–1.08)	0.582	0.93 (0.82–1.07)	0.304	0.97 (0.90–1.03)	0.305
Myocardial infarction	Ref	0.97 (0.94–1.01)	0.145	0.99 (0.95–1.04)	0.726	1.00 (0.97–1.02)	0.690
Peripheral artery disease	Ref	0.98 (0.93–1.03)	0.379	0.97 (0.91–1.03)	0.282	0.98 (0.95–1.01)	0.277

Adjusted for age, sex, assessment centres, top 10 genetic principal components, and genotyping array. Statistical significance was defined as Bonferroni-corrected threshold of $P < 4.2E-03$ (0.05/12).
CI, confidence interval; CVD, cardiovascular disease; GRS, genetic risk score; OR, odds ratio.

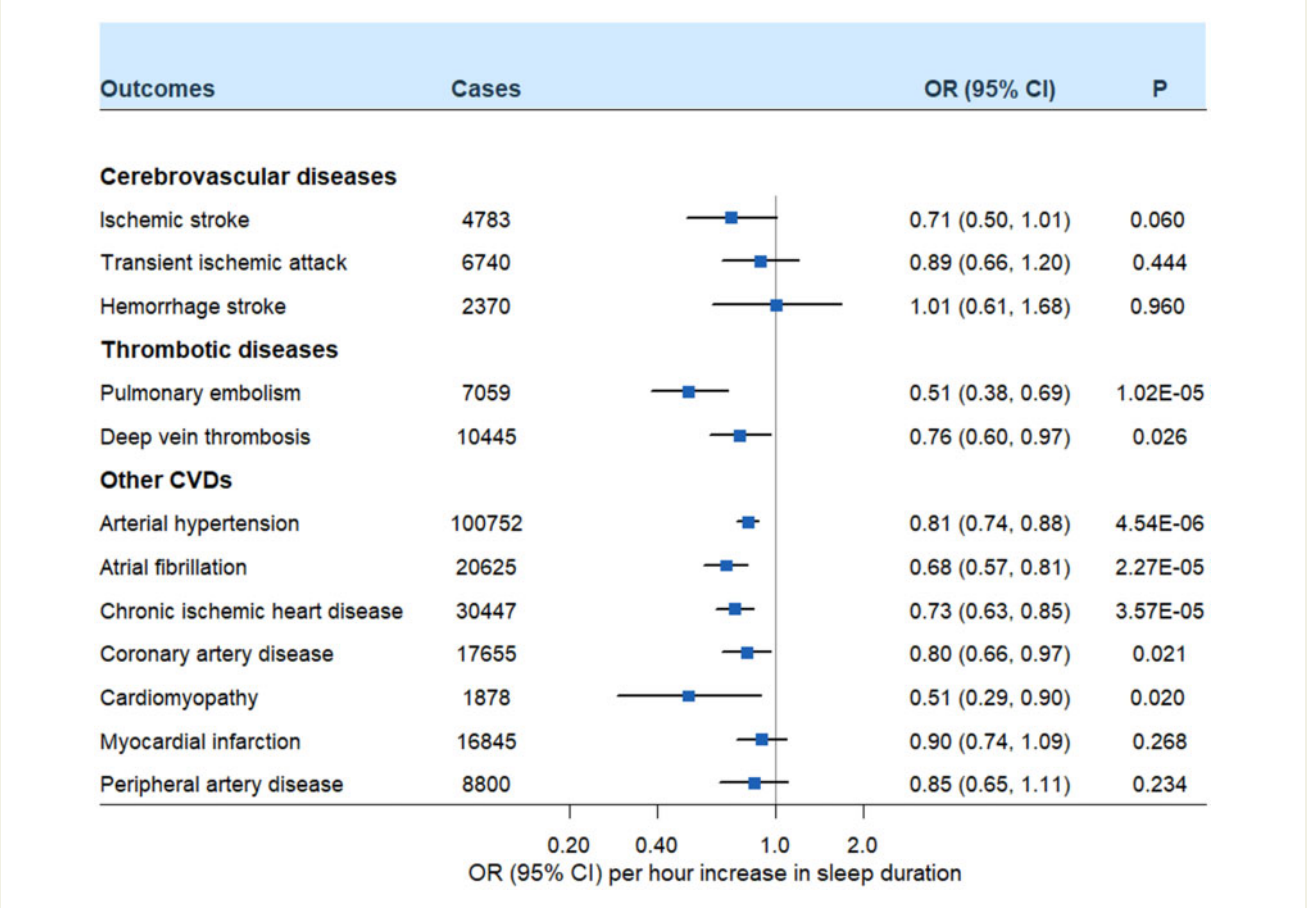


Figure 1 Linear Mendelian randomization estimates for the associations of genetically predicted sleep duration with 12 cardiovascular diseases in UK Biobank. Odds ratios were estimated by two-stage least squares regression method with adjustment for age, sex, assessment centre, top 10 genetic principal components, and genotyping array in both stages. The values represent genetically predicted 1 h increase in sleep duration resulting in an average change in the outcome. Statistical significance was defined as Bonferroni-corrected threshold of $P < 4.2E-03$ (0.05/12).

change markedly. The OR estimates obtained from the weighted median method were similar to those of the main analyses but with a relatively lower precision. The MR-Egger analysis revealed consistent estimates and suggested no directional pleiotropy for the CVD outcomes (Supplementary material online, *Table S10*).

Nonlinear Mendelian randomization analyses of sleep duration with 12 cardiovascular diseases

Nonlinear MR analyses were performed with a piecewise linear method using three strata according to the residual variation of sleep duration. The results indicated L-shaped associations between genetically predicted continuous sleep duration and the risks of arterial hypertension (Cochran Q $P = 9.96E-14$; Quadratic test $P = 7.22E-13$), coronary artery disease (Cochran Q $P = 1.90E-07$; Quadratic test $P = 2.15E-08$), chronic ischaemic heart disease (Cochran Q $P = 1.34E-06$; Quadratic test $P = 2.21E-06$), and myocardial infarction (Cochran Q $P = 9.52E-08$; Quadratic test $P = 1.28E-06$). This suggested a better fitting with the nonlinear model than the linear model (Supplementary material online, *Figure S3*). The LACE estimates suggested causal adverse effects of genetically predicted sleep duration on the risks of several CVDs in the short sleep duration strata but not in the long sleep duration strata (Supplementary material online, *Figure S3*).

Genetically predicted short and long sleep durations with the risk of 12 cardiovascular diseases

Figure 2 shows strong evidence of the adverse causal effects of having a genetically predicted short sleep duration on a broad range of CVDs, including arterial hypertension, coronary artery disease, myocardial infarction, chronic ischaemic heart disease, and pulmonary embolism ($P < 0.001$), and suggestive evidence for atrial fibrillation ($P < 0.05$). In contrast, there was no evidence on the adverse causal effects of genetically predicted long sleep duration on these CVDs (*Figure 2*). In the sensitivity analyses, the weighted median estimates

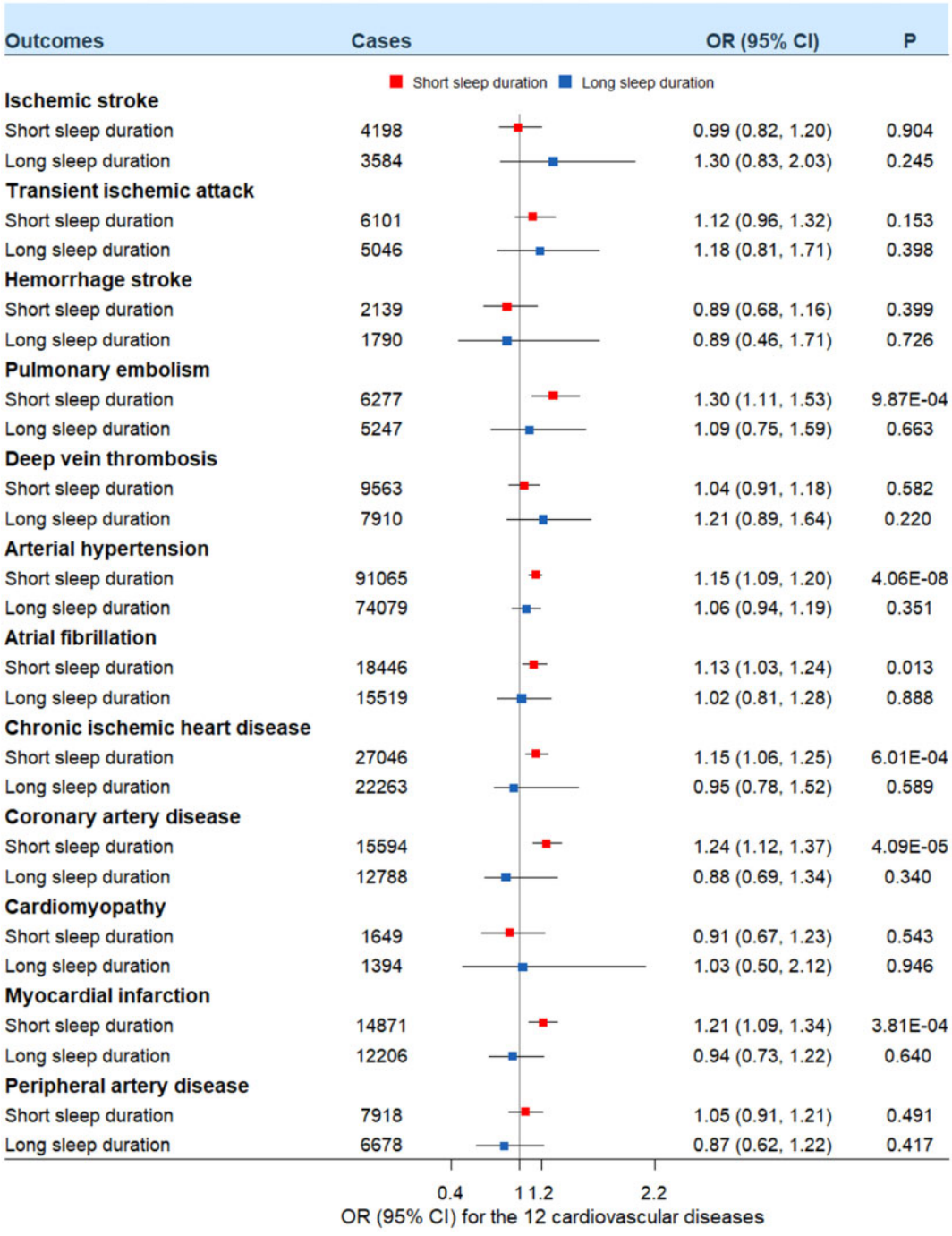


Figure 2 Mendelian randomization estimates for genetically predicted short and long sleep durations with 12 cardiovascular outcomes in UK Biobank. Odds ratios for genetically predicted short or long sleep duration were estimated by regression weighted sleep genetic allele score with cardiovascular disease risk using individual-level data from UKB, with adjustment for age, sex, assessment centre, top 10 genetic principal components, and genotyping array. The values reflect the increase in cardiovascular disease risk associated with per doubling changes in the odds of genetically predicted short or long sleep duration. Statistical significance was defined as Bonferroni-corrected threshold of $P < 4.2E-03$ (0.05/12).

showed the same directions as those in the main analyses, and the MR-Egger regression analyses did not find any pleiotropy (Supplementary material online, *Table S11*). The radial MR method identified one outlier each for coronary artery disease, chronic ischaemic heart disease, and myocardial infarction and five outliers for arterial hypertension (Supplementary material online, *Figure S4*). The

pleiotropic effects of these outliers are shown in Supplementary material online, *Figure S5*. After excluding these outliers, the OR estimates for arterial hypertension (1.15; 95% CI 1.09–1.22, $P = 4.17E-7$), coronary artery disease (1.29; 95% CI 1.16–1.43, $P = 3.78E-06$), myocardial infarction (1.25; 95% CI 1.12–1.39, $P = 5.78E-05$), and chronic ischaemic heart disease (1.12; 95% CI 1.02–1.21, $P = 9.69E-03$) did not change markedly. For the long sleep duration, the MR-Egger analyses detected potential pleiotropy for the outcomes of deep vein thrombosis and cardiomyopathy, but the results in the main and sensitivity analyses trended towards the null hypothesis for all CVDs (*Figure 2* and Supplementary material online, *Table S12*).

Discussion

In the current study, we found that unweighted GRS of self-reported sleep duration is associated with a lower risk of 4 out of 12 CVDs tested in this study (i.e. arterial hypertension, atrial fibrillation, pulmonary embolism, and chronic ischaemic heart disease). The linear MR analyses further provide evidence that genetically predicted sleep duration is a potentially causally factor of these four CVDs. Complementary analyses provided further evidence of adverse effects of genetically predicted short sleep duration on the risks of 5 out of 12 CVDs (*Graphical abstract*), with three overlapping with four CVDs found in linear association. However, genetically predicted long sleep duration was not adversely associated with any CVD. Nonlinear MR analyses demonstrated an L shaped rather than a U-shaped association between sleep duration and several CVDs. These analyses from different angles consistently suggest that genetically predicted short sleep duration is a potentially causal risk factor for common CVDs, while genetically predicted long sleep duration is unlikely to be a causal risk factor for CVDs.

Many conventional observational studies have confirmed that short sleep duration is longitudinally associated with increased risks of many CVDs.[6–8] Previous MR studies conducted by Daghlas *et al.*[20] and Gao *et al.*[31] have also suggested that short sleep duration may be a potential causal risk factor for some CVDs, such as myocardial infarction and coronary artery disease. Nevertheless, recent MR studies reported inconsistent results on the causal association between sleep duration and coronary heart disease.[22,23] One of the reasons may be due to the fact that there were fewer genetic variants of sleep duration in these studies than that of the previous two studies,[20,31] which may have decreased their statistical power with potential false negative results. In the current study, we employed the same set of genetic variants as Daghlas *et al.*[20] and Gao *et al.*[31] and found consistent evidence for the causal adverse effects of genetically predicted short sleep duration on a broad range of CVDs, including myocardial infarction and coronary artery disease. Larsson and Markus[32] found that genetic liability to insomnia is associated with a modest increased risk of four CVDs by using a two-sample MR study. Although clinical presentation of insomnia and short sleep duration is different, it seems that they influence cardiometabolic health through some common pathophysiological mechanisms, including dysfunction of the sympathetic nervous system, acceleration of metabolic diseases and atherosclerosis, increased inflammation, and cardiac dysfunction.[11,12,33,34] Recent studies also suggested that extended sleep duration could improve individuals' cardiovascular health, especially in college students or prehypertension participants, who are usually deprived of sleep.[35,36] Therefore, increasing sleep duration among short sleepers might be a promising strategy to reduce CVD risk.

Although long sleep duration has been found to have stronger associations with CVDs than short sleep duration,[16] it has long been a debate whether long sleep duration is a risk to one's health or is simply a surrogate marker of risk. One of the key reasons is that there is a lack of clear experimental evidence to support long sleep duration as a harmful effect to cardiovascular system, especially in healthy participants.[13,14] On the other hand, long sleep duration is closely associated with a number of risk factors of poor health status, such as depression, antidepressant use, poor sleep quality, low socioeconomic status, unemployment, and sedentary lifestyles.[9,17,37] The current study has provided suggestive evidence that genetically predicted long sleep duration is unlikely to be causally associated with the risks of CVDs. Since many aforementioned conditions can lead to longer sleep, the association between long sleep duration and CVD reported in observational studies may reflect a reverse causality or residual confounding.[38] Therefore, it is clinically advisable to look out for underlying morbidities (e.g. depression and sleep apnea) rather than a direct recommendation of reducing sleep duration for people with long sleep duration.

Because of the limited number of SNPs associated with long sleep duration, it may give rise to the concern of weak instrumental variables. We have considered this concern in our analyses and believe that the potentially weak instrumental variable is not likely to influence our conclusions. First, it has been demonstrated that any potential bias of weak instrumental variables in one-sample analysis will be towards the direction of the observational association between risk factor and outcome, rather than the null hypothesis.[39] Second, in the case of a null causal effect, a higher *F*-statistic of SNP exposure (i.e. >10) suggests less bias.[39] In the present study, the estimated F-statistic for long sleep duration was 294.7. Therefore, the bias, if any, shall be very limited. Third, the causal estimates of long sleep duration on most CVDs were largely consistent across different MR methods when using either weighted allele scores[20] or summarized data.[40]

Strengths and limitations

Compared with previous observational studies,[3,5,41] we used a MR study design to assess the causal associations between genetically predicted sleep duration and a wide range of CVDs in the same cohort. This design could minimize the potential biases due to confounding and reverse causality in the observational studies. On the other hand, the statistical power is relatively larger in the present study than previous MR studies,[20,22,23] benefiting from a larger sample size and more genetic variants. Another strength is that we used a nonlinear MR method to characterize the shape of causal associations between genetically predicted sleep duration and CVDs. Consistent evidence between linear and nonlinear MR supports the causal adverse effects of genetically predicted short sleep duration on many CVDs.

Some limitations should be noted when interpreting our findings. First, sleep duration was self-reported rather than objectively measured, such as by using polysomnography (PSG), but PSG is not usually feasible in a large cohort study. Previous studies have suggested that there is a moderate correlation between self-reported and objective

measured sleep duration.[42] In addition, habitual self-reported sleep duration seems rather stable within adult individuals across time,[10,43] which suggests that baseline sleep duration may represent a long-term exposure to a certain extent. Second, the genetic variants were associated with some confounders, such as body mass index, Townsend deprivation index, and education level of each participant, and might be affected by pleiotropy. However, the results were broadly consistent with those of the main analyses after adjusting for potential confounders. Third, the statistical powers of some outcomes (e.g. haemorrhagic stroke and transient ischaemic attack) are relatively low, which may raise the concern of false negatives. Fourth, the one-sample design is easily influenced by weak instrument bias. However, the *F*-statistic suggests a small magnitude of bias due to sample overlap. Fifth, although there is a lack of external validation of the GRS in the current study, a recent study with an independent clinical cohort from the USA using the same set of 78 SNPs has verified the association between the GRS and self-reported sleep duration with an apparently larger variance of self-reported sleep duration than UKB.[19] Finally, the selection criteria of the UKB tended to include relatively healthy participants aged 40–69 years at baseline, and this may limit the generalizability of our findings.

Conclusions

In this study, both linear and nonlinear MR analyses suggest that genetically predicted short sleep duration is associated with modest increased risks of most CVDs. However, genetically predicted long sleep duration is not associated with the risk of most CVDs. Therefore, the extension of sleep may benefit cardiovascular health for those people with sleep loss. For those people with long sleep duration, reducing sleep duration *per se* is unlikely to improve cardiovascular health.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Acknowledgements

This research has been conducted using the UKB Resource under Project Number 58082. We would like to thank Mr Xiaoyu Wang, Ms Shanshan Wang, and Ms Binbin Han from the Department of Cardiology, Heart Center, The First Affiliated Hospital of Xinxiang Medical University for their help in data acquisition and cleaning. We would also like to express our great appreciation to Prof. Suhua Chang from the Institute of Mental Health, Key Laboratory of Mental Health and Peking University Sixth Hospital for her help in interpreting results from MR analyses.

Funding

This work was supported by the National Natural Science Foundation of China (grant nos. 81801315 and U1904159), the 'Impact Postdoctoral Fellowship Scheme of The Chinese University of Hong Kong' (SZA), and the start-up fund for excellent scholar from the Guangdong Provincial People's Hospital (HJZ).

Ethical approval

UKB has received ethical approval from the UK National Health Service's National Research Ethics Service (ref 11/NW/0382).

Data sharing

Individual-level data from the UK Biobank are not publicly available due to their policy, but the data will be made available after the application of UK Biobank (https://www.ukbiobank.ac.uk/).

Conflict of interest: Y.-K.W. reports personal fees from Delivering a lecture—Eisai Co. Ltd and personal fees from Sponsorship from Lundbeck HK Ltd, outside the submitted work. The other authors declare that there is no conflict of interest.

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Translational perspective

This study employed both linear and nonlinear Mendelian randomization (MR) analyses to estimate the associations between genetically predicted sleep duration and a series of cardiovascular diseases (CVDs). The MR results suggested that genetically predicted short sleep duration is potentially causally associated with increased risks of several CVDs, including arterial hypertension, pulmonary embolism, coronary artery disease, myocardial infarction, and chronic ischaemic heart disease. These results have suggested that increasing sleep duration among short sleepers shall serve as a preventive strategy to reduce CVD risk. However, genetically predicted long sleep duration is unlikely to be a potential causal risk factor for most CVDs. These findings suggest that simply reducing sleep *per se* is not likely to improve cardiovascular health in people with long sleep duration.