Causal association between sleep traits and diabetic nephropathy

Keywords
sleep, diabetic nephropathy, causality, Mendelian randomization

Abstract

Introduction
To explore the causal association between genetically predicted sleep traits (chronotype, sleep duration, short sleep duration, long sleep duration, insomnia, daytime sleepiness, and sleep apnea syndrome) and diabetic nephropathy (DN).

Material and methods
A two-sample Mendelian randomization (MR) design was used to analyze summary data from genome-wide association studies of sleep traits and DN. The main analysis was conducted using the inverse variance weighted (IVW) method, and robustness was tested using the weighted median, weighted mode, and MR-Egger regression methods. Heterogeneity was detected using Cochran's Q-test, horizontal pleiotropy using the MR-Egger regression method, potential outliers using MR-PRESSO, and single-nucleotide polymorphisms driving the results using a leave-one-out analysis.

Results
The genetic prediction results indicated no statistically significant associations between the sleep traits and DN (all IVW P>0.05). However, the weighted median analysis showed a possible causal association between long sleep and DN (OR < 0.01, 95%CI: 0-0.70, P=0.04) and a borderline possible causal association between sleep apnea syndrome and DN (OR= 2.50, 95%CI: 0.99-6.35, P=0.05). Cochran's Q-test indicated possible heterogeneity for the sleep duration analysis (P=0. 01), but no horizontal pleiotropy or outliers were detected (all P>0.05).

Conclusions
This MR analysis suggested no causal associations between the sleep traits (chronotype, sleep duration, short sleep duration, long sleep duration, insomnia, daytime sleepiness, and sleep apnea syndrome) and DN. Further in-depth research is needed to examine the relationship between sleep and DN.

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Running title: MR: sleep and diabetic nephropathy

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Conclusion: This MR analysis suggested no causal associations between the sleep traits (chronotype, sleep duration, short sleep duration, long sleep duration, insomnia, daytime

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Keywords: sleep; diabetic nephropathy; Mendelian randomization; causality.

INTRODUCTION

Diabetic nephropathy (DN) is a microvascular complication of diabetes mellitus. DN may progress to chronic kidney disease (CKD) and is a major cause of end-stage kidney disease [1]. Diabetic kidney disease is a distinct clinical-pathologic entity clinically presenting as microalbuminuria or macroalbuminuria, increased arterial blood pressure, and a distinct morphologic pattern of injury on renal biopsy [1]. Approximately 20%-40% of patients with diabetes develop diabetic kidney disease [2]. The risk factors for diabetic kidney disease include genetic factors, smoking, duration of diabetes, early onset of diabetes, poor glycemic control, obesity, hypertension, proteinuria, dyslipidemia, and older age [1]. Sleep is a complex, active state of unconsciousness produced by the body, during which the brain is in a relative state of rest and is reactive primarily to internal stimuli. The exact purpose of sleep has not been fully elucidated, but theories attempting to identify why we sleep have been proposed: the inactivity theory, the energy conservation theory, the restoration theory, and the brain plasticity theory [3]. The impact of DN on sleep quality is fairly well documented [4]; however, whether sleep can influence the occurrence of DN remains poorly understood, although accumulating evidence suggests that obstructive sleep apnea (OSA) could be a risk factor for DN or be associated with DN [5]. Indeed, a parallel increase in the prevalence of OSA, T2DM, and DN has been observed in India and China [6]. Short or long sleep duration [7] and OSA [6] have been associated with DN, but whether genetically predicted sleep parameters are associated with a genetic predisposition to DN remains to be determined. Furthermore, treating OSA with continuous positive airway pressure does not improve DN parameters [8].

Data on millions of single-nucleotide polymorphisms (SNPs) and their related phenotypes have been provided in recent decades by genome-wide association studies (GWASs), revolutionizing the field of genetics and medicine, especially for complex traits and diseases [9]. The Mendelian randomization (MR) methodology uses common genetic variants as instrumental variables for various environmental exposures. It allows the exploration of possible causal associations between these exposures and outcomes. Two-sample MR uses the associations between SNPs and exposure and between SNPs and outcomes from different GWASs to combine them into a single analysis to estimate causality. Under key assumptions, MR reduces the impact of reverse causation and confounding that often substantially impede or mislead the interpretation of epidemiological studies [9].

Therefore, this study aimed to explore the causal association between genetically predicted sleep traits and DN using the MR methodology.

MATERIALS AND METHODS

Study design

The overall design of this two-sample MR study is illustrated in **Figure 1**. First, we identified and curated large-scale, publicly available GWAS summary statistics for seven distinct sleep traits (exposures) and DN (outcome). Second, for each sleep trait, we selected independent single nucleotide polymorphisms (SNPs) that were strongly associated with the exposure ($P < 5 \times 10^{-8}$) and were not in linkage disequilibrium ($r^2 < 0.001$ within a 10 Mb window) to serve as instrumental variables (IVs). Third, we performed the primary causal estimation using the inverse-variance weighted (IVW) method. Finally, to ensure the robustness of our findings, we conducted a comprehensive suite of sensitivity analyses, including the weighted median, weighted mode, MR-Egger regression, MR-PRESSO, and leave-one-out analyses, to detect and account for potential horizontal pleiotropy and heterogeneity.

This study was conducted using exclusively publicly available summary-level data. The original GWASs were approved by ethical committees and were performed according to the tenets of the Declaration of Helsinki. Hence, no additional ethical approval was necessary.

The validity of our MR study hinges on three key assumptions: 1) the relevance assumption, which posits that the selected IVs are robustly associated with their corresponding sleep trait; 2) the independence assumption, which requires that there are no unmeasured confounders of the association between the IVs and DN; and 3) the exclusion-restriction assumption (or no horizontal pleiotropy), which states that the IVs affect DN only through their effect on the sleep trait and not via any independent biological pathways [9].

Data source

The GWAS data for DN were from the FinnGen project (https://www.finngen.fi/en). The dataset contains data on 3283 patients with DN and 181,704 controls (16,380,336 SNPs), all from Finland.

The chronotype dataset was from a published study [10]. It contains data on 403,195 individuals from the United Kingdom (UK). The sleep duration data were from 446,118 individuals from the UK [11]. That dataset contains information about long sleep duration 339,926 individuals) and short sleep duration (446,118 individuals) [11]. The data about sleep apnea syndrome were from 25,008 cases and 337,630 controls from Canada, the United States of America (USA), Finland, the UK, and Australia [12]. The datasets on insomnia (from Europe) [13] and daytime sleepiness (from the USA) [11] were from published studies (**Table S1**).

Instrumental variable selection

The IVs included in this study had to meet the following criteria. First, the SNPs significantly associated with the sleep traits were screened, with a threshold of $P<5\times10^{-8}$ [14]. Then, the SNPs with a minimum minor allele frequency (MAF) of >0.01 were selected. Finally, SNPs showing linkage disequilibrium (LD) were filtered out based on $R^2<0.001$ and a window size of 10,000 kb. In cases where a selected IV for an exposure was not present in the outcome summary data, SNPs with high LD ($R^2>0.8$) were sought as proxy SNPs for replacement. The F-statistic was calculated for each SNP in the IV to assess IV strength and identify potential weak instrument bias between IVs and exposure factors. The formula was $F=R^2\times(N-2)/(1-R^2)$. An F-statistic >10 was required to mitigate weak instrument bias [15].

Mendelian randomization analysis

The primary method for MR analysis to assess the causal association between exposure and outcome risks in this study was the inverse variance weighted (IVW) method [16]. The results were provided as odds ratios (ORs) and 95% confidence intervals (CIs). If the IVW results were statistically significant, their robustness was tested using complementary MR methods, including the MR-Egger [17], weighted median [18], and weighted mode [19]. All analyses were carried out using the "TwoSampleMR" package in R 4.0.5 (The R Project for Statistical Computing, www.r-project.org). The analyses were visualized using scatter and forest plots.

Sensitivity analysis

Heterogeneity was detected using Cochran's Q test; $P>0.05$ indicated low heterogeneity, suggesting that the estimates among IVs were randomly distributed and had little impact on the IVW analysis [20]. Funnel plots were generated to visualize heterogeneity. Horizontal pleiotropy was detected using the MR-Egger regression method; an intercept near 0 or $P>0.05$ indicated the absence of pleiotropy [20]. The MR pleiotropy residual sum and outlier (MR-PRESSO) analysis was used to detect potential outliers, indicated by $P<0.05$, and re-estimate the causal associations after outlier removal [20]. A leave-one-out analysis was used to determine whether single SNPs drove the results; the leave-one-out analysis was shown as forest plots.

RESULTS

Instrument variable selection

In this study, when performing MR analyses with chronotype, sleep duration, short sleep duration, long sleep duration, insomnia, daytime sleepiness, and sleep apnea syndrome as exposures, 122, 70, 26, 10, 13, 33, and 5 IVs were identified, respectively. The mean F-statistics for these IVs were 45.67, 40.67, 34.35, 31.91, 41.19, 42.45, and 43.43, respectively. The minimum F-statistics ranged from 29.02 to 29.88 for the different exposures, while the maximum values extended from 52.98 to 168.52. No SNPs were unmatched in the summary data for chronotype, short sleep duration, insomnia, daytime sleepiness, and sleep apnea syndrome-related IVs. In contrast, two SNPs were unmatched for long sleep duration, without proxy SNPs being identified. The R^2 values, indicating the proportion of variance explained by the IVs for each trait, were 0.01, 0.006, 0.002, 0.001, 0.004, 0.003, and <0.001, respectively, for the different exposures. The selection of the IVs is summarized in **Table S2**. All F-values were >10, indicating the absence of weak instrumental bias (**Table S3**).

Mendelian analysis results

Genetic prediction results indicated no statistically significant associations between the sleep traits and DN (all IVW P>0.05) (**Table 1** and **Figure 2** and **Figure 3**). The weighted median analysis showed a possible causal association between long sleep and DN (OR=0, 95%CI: 0-0.70, P=0.04) and a borderline possible causal association between sleep apnea syndrome and DN (OR=2.50, 95%CI: 0.99-6.35, P=0.05). However, such results must be taken cautiously since the main MR analysis is IVW.

Sensitivity analysis

Heterogeneity among IVs can bias the results. Cochran's Q-test indicated possible heterogeneity for the sleep duration analysis (P=0.01) (**Table S4** and **Figure S1**). The third key assumption for the validity of MR analyses is the absence of horizontal pleiotropy. MR-Egger regression suggested the absence of horizontal pleiotropy (all P>0.05) (**Table S4**). MR-PRESSO did not detect outliers (**Table S5**), suggesting the robustness of the results. The leave-one-out analysis suggested that no single SNP drove the results (**Figure S2**), also suggesting robustness.

DISCUSSION

This two-sample MR study explored the causal association of sleep traits, including chronotype, sleep duration, short sleep duration, long sleep duration, insomnia, daytime sleepiness, and sleep apnea syndrome, with DN. The results suggested no causal associations between sleep traits and DN based on the primary IVW analyses. Still, the weighted median analysis showed a possible causal association between long sleep and DN and a borderline possible causal association between sleep apnea syndrome and DN.

DN affects the sleep quality of the patients, but the relationship is complex and is influenced by physical and psychological factors [4]. The present MR study did not delve into that association. On the other hand, whether sleep can influence the occurrence of DN remains poorly understood. Nevertheless, poor sleep quality is associated with poor health outcomes. Indeed, short and long sleep durations are associated with overall mortality (absolute increases in mortality of 12% and 39%, respectively) and cognitive decline [21]. Compared with 7 h of sleep, a 1-h decrease in sleep duration was associated with an 11% increase in the risk of cardiovascular heart disease, while a 1-h increase in duration was associated with a 7% increase [22]. In addition, compared with 7 h of sleep, a 1-h decrease in sleep duration was associated with a 9% increase in the risk of type 2 diabetes mellitus, while a 1-h increase in duration was associated with a 14% increase [23]. Short sleep was also associated with falls, accidents, and injuries, incident obesity, and incident hypertension [21]. Although those previous studies did not examine DN, diabetes is a prerequisite for DN diagnosis, and obesity and hypertension are risk factors for DN [1]. The intermittent hypoxia observed in sleep apnea is also associated with a systemic proinflammatory state [24] that can contribute to the occurrence of DN. Sleep disturbances

are also associated with systemic inflammation [25]. Indeed, inflammation is involved in the pathogenesis of DN [26]. Furthermore, OSA can influence the blood glucose levels [27].

Still, the present study showed no causal associations between sleep traits and DN, based on the IVW results. The weighted median analysis suggested the possibility of causal associations between long sleep duration and DN and between sleep apnea and DN, but those results were not observed in the IVW analysis. They must be taken with caution, but suggest the possibility of an association that might warrant additional study. The pathogenesis of many diseases is influenced by a combination of genetic and environmental factors [28]. Even though genetic factors are a major determinant of disease risk, environmental factors also play an important role [28]. Therefore, in the case of negative genetic results, as is the case here, examining whether there is an interaction between environmental factors and the exposures and outcomes, and how these factors affect the results, is necessary. Unfortunately, GWAS datasets do not contain such information. Longitudinal studies would be necessary to look into those points. Therefore, negative MR results do not mean that there is no link between the exposures and the outcomes, but that the genetically predicted risk of the exposure is not associated with the genetically predicted risk of the outcome. The absence of association can be because the genetic variation is insufficient to model the effect of exposure on outcome adequately. It is a known limitation of MR studies, especially where genetic variants have small or weaker effects on exposure.

This study had several strengths. It used a two-sample MR design and GWAS data from tens of thousands of individuals to evaluate the causal association between sleep traits,

OSA, and DN. On the other hand, the study also had limitations. First, the GWAS data in the present study were from European, North American, and Australian populations (i.e., mainly of European ancestry), and their generalizability to other populations remains unknown. Second, some participants could have been included in both the exposure and the outcome, but it is impossible to determine the exact extent of the phenomenon. Still, using stringent statistics should minimize the impact of eventual overlap [29]. The selection of IVs can influence causal associations, and while the present study applied robust selection criteria, the inherent genetic architecture of the traits might limit the strength or number of available IVs. Third, MR studies assess the effect of lifelong genetic predisposition to an exposure, which may not fully capture the impact of acquired or fluctuating sleep patterns on DN risk. Finally, negative MR results indicate that the genetically predicted risk of the exposures is not associated with the genetically predicted risk of the outcome, which can occur if the genetic variation is insufficient to model the effect of exposure on outcome adequately, a known limitation where genetic variants have weaker effects on exposure.

In conclusion, the present MR analysis suggested no causal associations between sleep traits (chronotype, sleep duration, short sleep duration, long sleep duration, insomnia, daytime sleepiness, and sleep apnea syndrome) and DN. Further in-depth research is needed to examine the relationship between sleep and DN.

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Figure legends:

Figure 1. Schematic representation of the Mendelian randomization analysis.

Figure 2. Mendelian randomization analysis of the causal association between sleep traits and diabetic nephropathy. Scatter plots for (A) chronotype, (B) sleep duration, (C) short sleep duration, (D) long sleep duration, (E) insomnia, (F) daytime sleepiness, and (G) sleep apnea syndrome.

Figure 3. Mendelian randomization analysis of the causal association between sleep traits and diabetic nephropathy. Forest plots for (A) chronotype, (B) sleep duration, (C) short sleep duration, (D) long sleep duration, (E) insomnia, (F) daytime sleepiness, and (G) sleep apnea syndrome.

Supplementary materials:

Figure S1. Mendelian randomization analysis of the causal association between sleep traits and diabetic nephropathy. Funnel plots for (A) chronotype, (B) sleep duration, (C) short sleep duration, (D) long sleep duration, (E) insomnia, (F) daytime sleepiness, and (G) sleep apnea syndrome.

Figure S2. Mendelian randomization analysis of the causal association between sleep traits and diabetic nephropathy. Leave-one-out forest plots for (A) chronotype, (B) sleep duration, (C) short sleep duration, (D) long sleep duration, (E) insomnia, (F) daytime sleepiness, and (G) sleep apnea syndrome.

Table S1. GWAS information for all outcomes and exposures

Table S2. Detailed information of instrumental variables used in MR analyses

Table S3. Summary of instrumental variables for each sleep trait

Table S4. Results of heterogeneity test and pleiotropy tests of instrumental variables

Table S5. Results of MR-PRESSO global test and outlier test

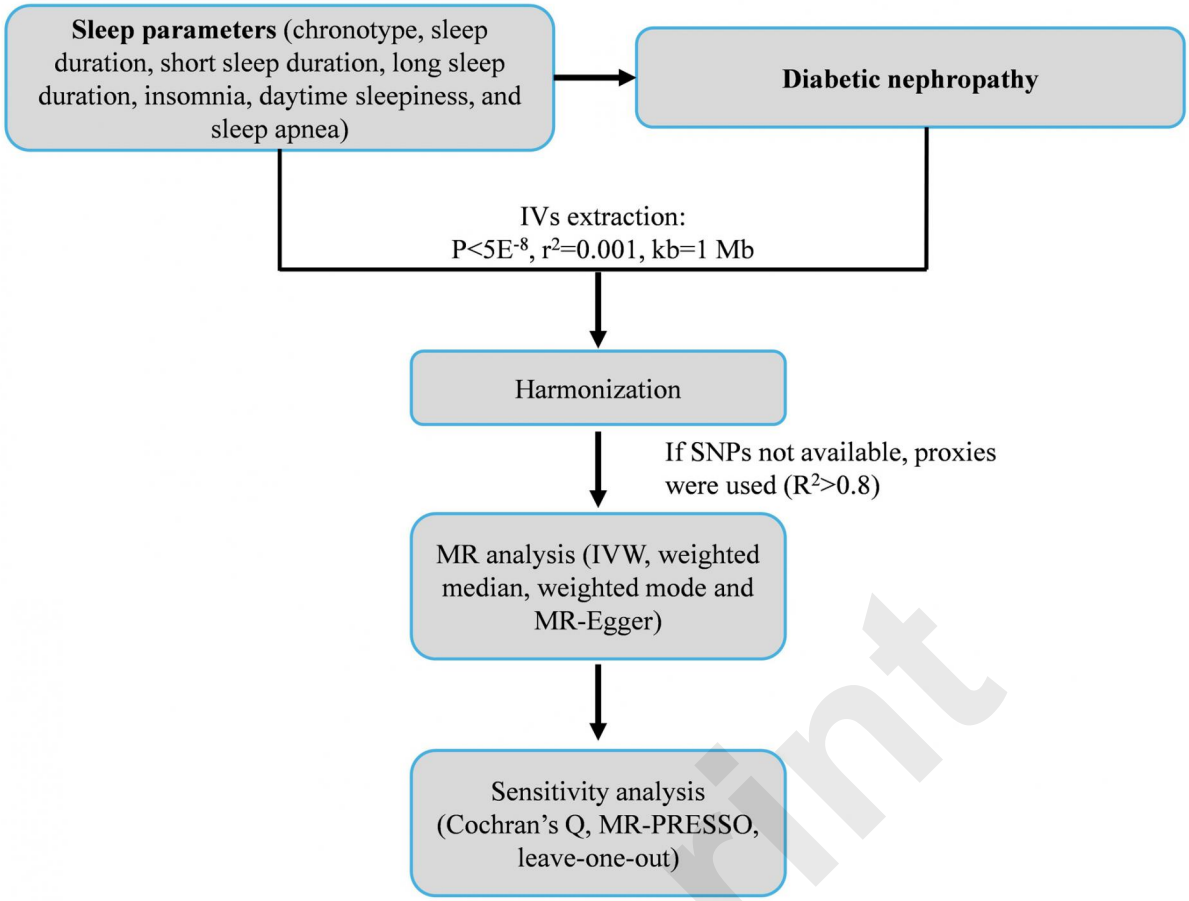
Sleep parameters (chronotype, sleep duration, short sleep duration, long sleep duration, insomnia, daytime sleepiness, and sleep apnea)
Diabetic nephropathy
IVs extraction:
P<5E-8, r2=0.001, kb=1 Mb
Harmonization
If SNPs not available, proxies were used (R2>0.8)
MR analysis (IVW, weighted median, weighted mode and MR-Egger)
Sensitivity analysis (Cochran's Q, MR-PRESSO, leave-one-out)

Table 1. Genetic prediction of the association between sleep characteristics and the risk of developing causal associations with diabetic nephropathy

Exposure	Outcome	N.SNPs	Methods	OR (95% CI)	P
Chronotype	Diabetic nephropathy	118	IVW	1.82 (0.91-3.65)	0.09
			MR-Egger	1.99 (0.22-17.87)	0.54
			Weighted median	1.68 (0.63-4.47)	0.3
			Weighted mode	1.24 (0.09-16.83)	0.87
Sleep duration		67	IVW	0.82 (0.48-1.39)	0.45
			MR-Egger	0.53 (0.07-4.26)	0.55
			Weighted median	0.57 (0.29-1.11)	0.1
			Weighted mode	0.48 (0.15-1.54)	0.22
Short sleep duration		24	IVW	1.19 (0.17-8.42)	0.86
			MR-Egger	21959.04 (0.66-728072219.24)	0.07
			Weighted median	3.58 (0.27-47.70)	0.33
			Weighted mode	15.81 (0.23-1074.40)	0.21

Long sleep duration	8	IVW	0.05 (0-30.86)	0.37
		MR-Egger	37572.36 (0-6154502976708.14)	0.32
		Weighted median	0 (0-0.70)	0.04
		Weighted mode	0 (0-5.39)	0.15
Insomnia	13	IVW	1.20 (0.76-1.89)	0.44
		MR-Egger	1.07 (0.29-3.96)	0.92
		Weighted median	1.31 (0.73-2.35)	0.36
		Weighted mode	1.39 (0.63-3.03)	0.43
Daytime sleepiness	32	IVW	0.45 (0.11-1.93)	0.28
		MR-Egger	3.26 (0-3151.51)	0.74
		Weighted median	0.91 (0.12-6.8)	0.92
		Weighted mode	1.12 (0.04-31.86)	0.95
Sleep apnea syndrome	5	IVW	1.86 (0.70-4.92)	0.21
		MR-Egger	9.57 (0.05-2009.71)	0.47
		Weighted	2.50 (0.99-6.35)	0.05

	median		
	Weighted mode	3.30 (0.99-11.03)	0.12

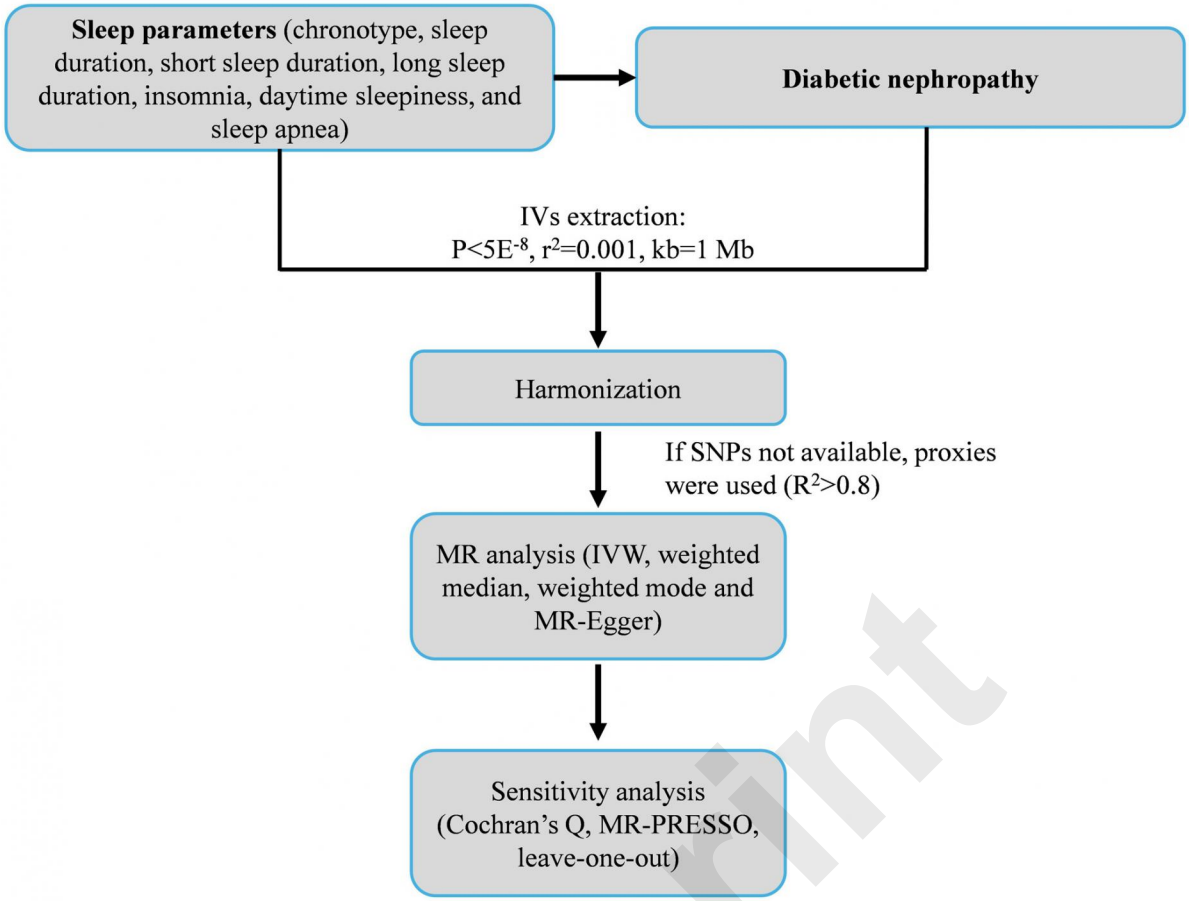

Figure 1. Schematic representation of the Mendelian randomization analysis.

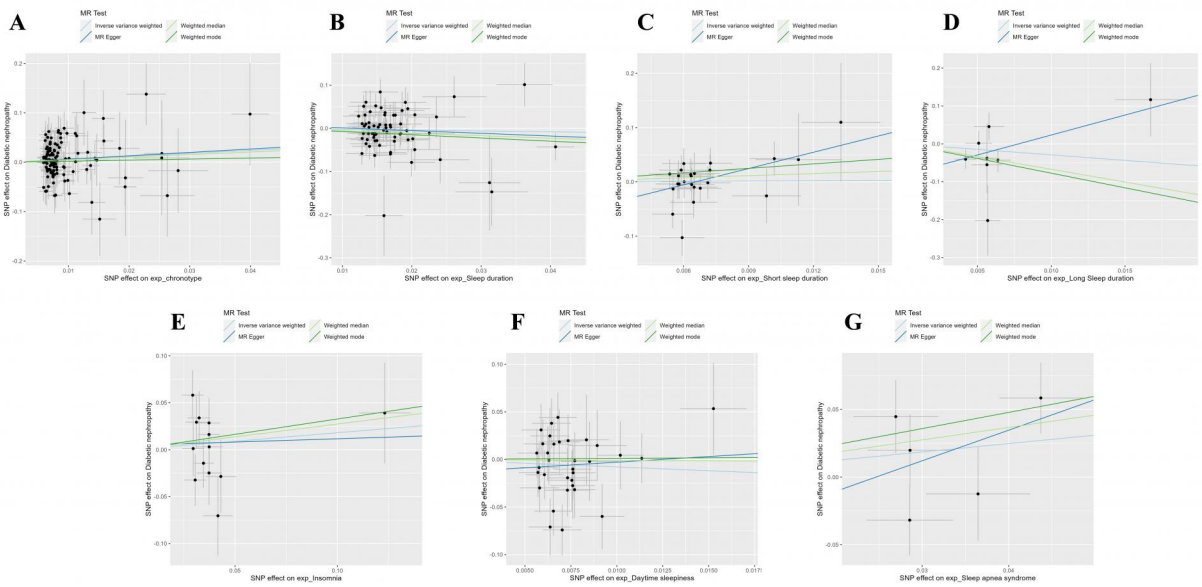

Figure 2. Mendelian randomization analysis of the causal association between sleep traits and diabetic nephropathy. Scatter plots for (A) chronotype, (B) sleep duration, (C) short sleep duration, (D) long sleep duration, (E) insomnia, (F) daytime sleepiness, and (G) sleep apnea syndrome.

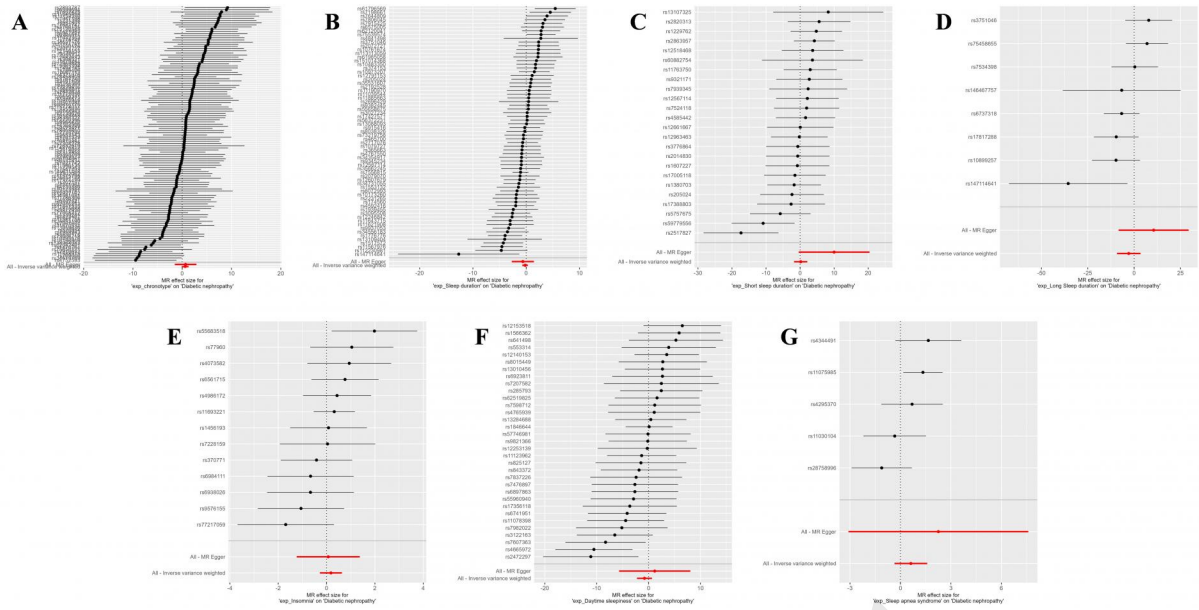

Figure 3. Mendelian randomization analysis of the causal association between sleep traits and diabetic nephropathy. Forrest plots for (A) chronotype, (B) sleep duration, (C) short sleep duration, (D) long sleep duration, (E) insomnia, (F) daytime sleepiness, and (G) sleep apnea syndrome.