



## SGLT2 inhibition, circulating proteins, and insomnia: A mendelian randomization study

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### ABSTRACT

**Background:** Sodium-glucose cotransporter 2 inhibitors (SGLT2i) initially emerged as oral antidiabetic medication but were subsequently discovered to exhibit pleiotropic actions. Insomnia is a prevalent and debilitating sleep disorder. To date, the causality between SGLT2 inhibitors and insomnia remains unclear. This study aims to evaluate the causality between SGLT2 inhibitors and insomnia and identify potential plasma protein mediators.

**Methods:** Using a two-sample Mendelian Randomization (MR) analysis, we estimated the causality of SGLT2 inhibition on insomnia and sleep duration. Additionally, employing a two-step and proteome-wide MR analysis, we evaluated the causal link of SGLT2 inhibition on 4907 circulating proteins and the causality of SGLT2 inhibition-driven plasma proteins on insomnia. We applied a false discovery rate (FDR) correction for multiple comparisons. Furthermore, mediation analyses were used to identify plasma proteins that mediate the effects of SGLT2 inhibition on insomnia.

**Results:** SGLT2 inhibition was negatively correlated with insomnia (odds ratio [OR] = 0.791, 95 % confidence interval [CI] [0.715, 0.876],  $P = 5.579 \times 10^{-6}$ ) and positively correlated with sleep duration ( $\beta = 0.186$ , 95 % CI [0.059, 0.314],  $P = 0.004$ ). Among the 4907 circulating proteins, diadenosine tetraphosphatase (Ap4A) was identified as being linked to both SGLT2 inhibition and insomnia. Mediation analysis indicated that the effect of SGLT2 inhibition on insomnia partially operates through Ap4A ( $\beta = -0.018$ , 95 % CI [-0.036, -0.005],  $P = 0.023$ ), with a mediation proportion of 7.7 %.

**Conclusion:** The study indicated a causality between SGLT2 inhibition and insomnia, with plasma Ap4A potentially serving as a mediator.

### 1. Introduction

Sodium-glucose cotransporter 2 inhibitors (SGLT2i), also known as gliflozins, initially emerged as oral antidiabetic medication for type 2 diabetes [1]. By inhibiting the SGLT2 protein, this class of medication can prevent the kidneys from reabsorbing glucose, leading to the excretion of excess glucose in the urine and a reduction in blood glucose levels [2]. However, beyond their hypoglycemic effects, the remarkable cardioprotective and renal protective properties of SGLT2i were soon revealed, resulting in their approval for the treatment of heart failure

(HF) and chronic kidney disease (CKD), regardless of the patient's diabetic status [3,4]. Encouragingly, SGLT2i also exhibits pleiotropic actions, suggesting potential benefits across various clinical conditions beyond the cardio-renal-metabolic spectrum. Several mechanistic and clinical data indicated that gliflozins could improve outcomes related to infections, malignancies, cognitive decline and more [5]. Emerging evidence suggests that SGLT2i may benefit patients with diverse disorders, including chronic obstructive pulmonary disease [6], cirrhosis with ascites [7], and chronic syndrome of inappropriate antidiuresis [8], highlighting the increasing focus on SGLT2i as non-cardiometabolic

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medications. To date, there have been no reports on the effect of SGLT2 inhibitors on insomnia. This article aims to investigate the potential relationship between SGLT2 inhibition and insomnia using Mendelian randomization (MR) analysis.

Insomnia is a common sleep disorder that affects a significant portion of the global population, with prevalence rates varying from 10 % to 48 % [9]. Insomnia disorder is characterized by difficulties in falling asleep, staying asleep, and waking up prematurely, accompanied by an inability to return to sleep [10,11]. Sleep is essential for memory consolidation, metabolism regulation, and clearing metabolic byproducts [12]. Insomnia can result in decreased cognitive function, impaired learning and work performance, and in more severe cases, increased susceptibility to infections and cardiovascular diseases [13,14]. Therefore, finding effective strategies for treating insomnia is of great importance.

Mendelian randomization (MR) analysis is a valuable and increasingly popular epidemiology approach. In MR, genetic variants, commonly single nucleotide polymorphisms (SNPs) from genome-wide association studies (GWAS), are employed as instrumental variables (IVs) to estimate potential causality between exposure and outcome traits [15]. Genetic variants are randomly allocated at the time of gamete formation and conception according to Mendel's laws, independently of potential confounding environmental factors and are not subsequently affected by outcomes [16,17]. Therefore, MR is less susceptible to confounding and reverse causation compared with traditional observational studies. Recently, MR analysis has been widely employed for drug repurposing [18]. Furthermore, with the advancements in high-throughput genomic and proteomic techniques in plasma, MR-based strategies have facilitated the discovery of potential therapeutic targets for numerous diseases [19].

In this study, we aim to estimate the causal relationship between SGLT2 inhibition and insomnia using a two-sample MR study. Additionally, we incorporate proteome-wide MR and mediation analyses to identify plasma proteins that mediate the effects of SGLT2 inhibition on insomnia.

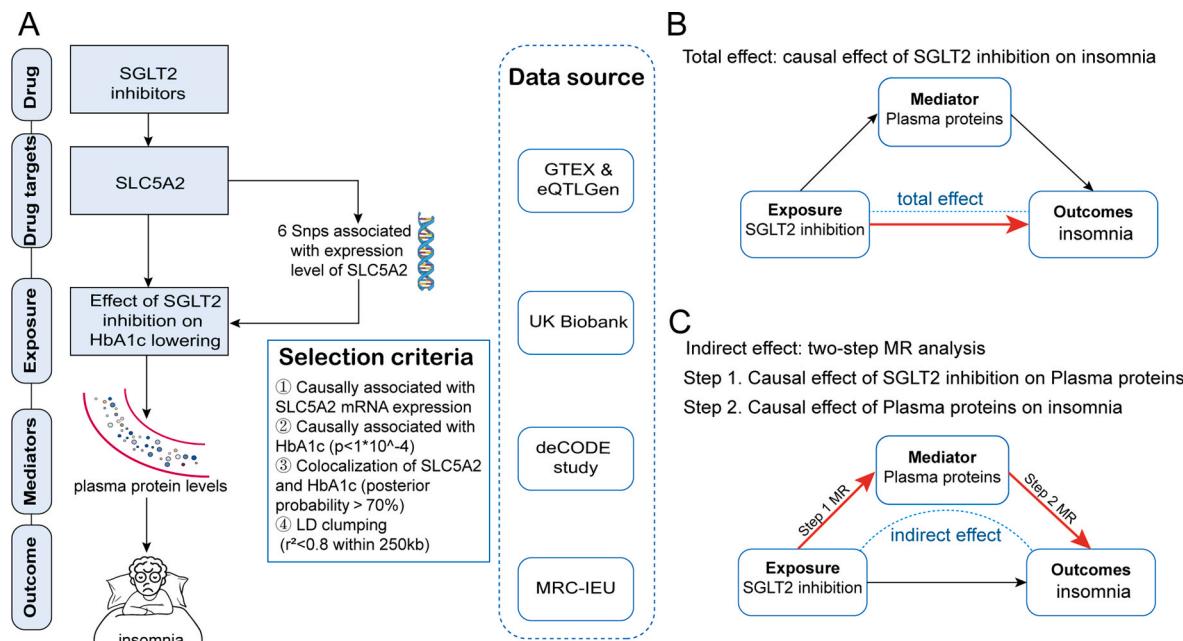
## 2. Methods

### 2.1. Study design

**Fig. 1A** illustrates the experimental framework design. Initially, a two-sample MR analysis was performed to investigate the causal relationship between SGLT2 inhibition and insomnia. (**Fig. 1B**). Following that, a two-step MR analysis was carried out to identify potential therapeutic targets that mediate the effects of SGLT2 inhibition on insomnia (**Fig. 1C**). The MR analysis is based on three key assumptions [20]: (1) the selected genetic variants are strongly linked to the exposure; (2) the selected genetic variants are independent of potential confounders; (3) the influence of chosen genetic variants on outcomes is solely mediated through the exposure variable. The study was reported following the Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization (STROBE-MR) guidelines [21], and the STROBE Checklist can be found in the Supplementary materials. For data sources, **Supplementary Table 1** provides the characteristics of the summary-level data derived from GWAS with ethical approval protocols as authorized by the respective original study [22].

### 2.2. Selection and validation for genetic predictors of SGLT2 inhibition

The selection of genetic variants that can proxy the effects of SGLT2 inhibition consisted of four rigorous steps, as described in previous studies [23–26]. First, we selected genetic variants within a 250-kilobase (kb) window around the *SLC5A2* gene (encoding SGLT2) and associated with mRNA expression levels of the *SLC5A2* gene in one or more of 50 tested tissues ( $p < 0.001$ , the threshold of significance within the genomic region) utilizing data from GTEx (Genotype-Tissue Expression,  $n \leq 706$ ) project [27] and eQTLGen Consortium (whole blood samples, sample size  $n \leq 31,684$ ) [28]. Second, according to previous MR studies, the relationship of individual *SLC5A2* variants with HbA1c levels was evaluated as a marker of reducing glucose levels via SGLT2 inhibition [29,30]. The HbA1c-associated variants were exclusively chosen from a subgroup of unrelated European individuals without diabetes in the UK Biobank dataset based on their genetic



**Fig. 1.** Study design and summary

A. The flowchart for investigating the effects of plasma proteins in mediating the effects of SGLT2 inhibition on insomnia (left). The criteria for SGLT2 inhibition instrument selection (middle). The GWAS data source (right). B. The flowchart for investigating the total effects of SGLT2 inhibition on insomnia. C. The flowchart for investigating the indirect effects of SGLT2 inhibition on insomnia mediated by plasma proteins, using a two-step MR.

relationship ( $n = 344,182$ ). Third, genetic colocalization analysis was employed to determine the presence of a shared causal variant within the *SLC5A2* region that influences the expressions of both *SLC5A2* and HbA1c, with a genetic colocalization probability threshold of 0.7. Finally, a conventional clumping procedure was implemented to remove variants with strong correlations exceeding the threshold of 0.8. Ultimately, six variants strongly associated with SGLT2 inhibition via HbA1c were retained for subsequent MR analysis (Supplementary Table 2).

### 2.3. Proteomic data source

The genetic associations with 4,907 proteins in the plasma proteome were derived from a large-scale pQTL (protein quantitative loci) research of 35,559 Icelanders [22]. According to the European 1000 Genomes Project reference panel, the IVs for each pQTL were obtained for MR analysis based on the following criteria:  $p < 5e-8$ ,  $r^2 < 0.001$ , MAF (minimum allele frequency)  $> 0.01$ , and without linkage disequilibrium within 10 MB [31].

### 2.4. Outcome data sources

Summary data on the genetic association with insomnia were acquired from the MRC-IEU consortium, consisting of 462,341 individuals of European descent. The Neale Lab, comprising 335,410 European individuals, provided summary statistics regarding the genetic correlation with sleep duration.

### 2.5. MR analysis

To find a statistically significant causal link, we used five different MR methods: random-effects inverse-variance weighted (IVW), MR Egger, weighted median, simple mode, and weighted mode. For exposures with a single SNP, we used the Wald ratio approach. The primary method utilized in MR analysis is the inverse-variance-weighted (IVW) method, which estimates the causal effect of exposure on outcomes by conducting a weighted average of the effect sizes obtained from instrumental variable estimates [32]. The weighted-median method was employed to assess causality in the presence of heterogeneity. This method allows for up to 50 % deviations from the MR analysis assumptions in instrumental variable estimates. In addition, scatter plots were used to illustrate the causal direction, and forest plots visually presented the effect size and statistical significance. Multiple analyses were assessed using the false discovery rate (FDR) criterion with a significance threshold set at  $P = 0.05$  [33]. We used R v.4.3.1 software (<https://www.r-project.org/>) and the “TwoSampleMR” package (<https://mrcieu.github.io/TwoSampleMR/>) to conduct MR analysis.

### 2.6. Validation of MR assumptions and sensitivity analysis

To fulfill the three fundamental assumptions of MR analysis. The F-statistic was used to evaluate the IVs' strength [34,35].  $F = \frac{(n-k-1)}{k} \times \frac{R^2}{1-R^2}$ ,  $n$  = sample size,  $k = 1$ , the coefficient of determination ( $R^2$ ) served as a metric to measure the proportion of variation explained by individual SNPs,  $R^2$  is calculated using the following formula :  $R^2 = \frac{2 \times \beta^2 \times EAF \times (1-EAF)}{2 \times \beta^2 \times EAF \times (1-EAF) + 2 \times SE^2 \times n \times EAF \times (1-EAF)}$ . IVs with an F-statistic less than 10 were excluded to mitigate potential result bias. We used the intercept P-value from MR Egger regression and the global test P-value from MR Pleiotropy RESidual Sum Outlier (MR-PRESSO) to assess pleiotropy. A P-value lower than 0.05 indicated the presence of horizontal pleiotropy associated with IVs. Heterogeneity was assessed using Cochran's Q statistic, with a P-value lower than 0.05 and an  $I^2$  value excess of 50 %, indicating the existence of apparent heterogeneity. The MR radial and MR-PRESSO methods were utilized for outlier detection and exclusion.

In the presence of heterogeneity, a random-effects IVW model was used, but in the absence of heterogeneity, a fixed-effects IVW model was used. A “leave-one-out” strategy was employed to assess the effect of each SNP on the overall causal estimate.

### 2.7. Mediation MR analysis linking SGLT2 inhibition with insomnia

A two-step MR analysis was conducted to study the potential mediation role of plasma proteins in the association between SGLT2 inhibition and insomnia. The mediation effect was assessed using the “product of coefficient” approach [36]. The percentage of the total effect mediated by plasma protein levels was determined by dividing the indirect influence ( $\beta_1 \times \beta_2$ ) by the total effect ( $\beta_3$ ). Here,  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  represent the effects of SGLT2 inhibition on plasma proteins, circulating proteins on insomnia, and SGLT2 inhibition on insomnia, respectively. A two-sample MR analysis was employed to generate effect estimates, and the “delta method” was used to calculate standard errors.

## 3. Results

### 3.1. Effect of SGLT2 inhibition on insomnia and sleep duration

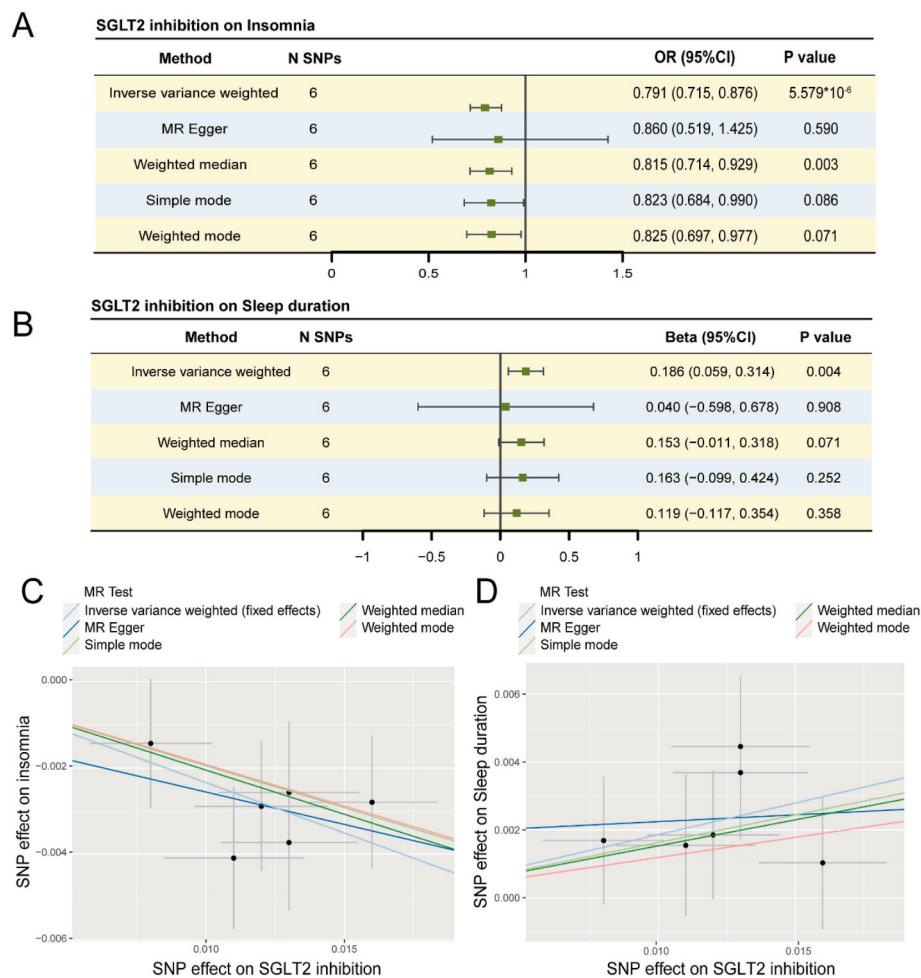
Six SNPs were identified as genetic predictors of SGLT2 inhibition (Supplementary Table 2). The F statistics for all SNPs were larger than 10, indicating that all SNPs were strong IVs. Our study revealed a negative association between SGLT2 inhibition and insomnia. Using the IVW method, the odds ratio (OR) for one-standard-deviation (SD) decrease in HbA1c resulting from SGLT2 inhibition was estimated to be 0.791 (95%CI [0.715, 0.876],  $P = 5.579 \times 10^{-6}$ ) (Fig. 2A, Supplementary Table 3). Other statistical methods consistently indicated similar estimate directions between SGLT2 inhibition and insomnia, thus corroborating the robustness of our main findings. Scatter plots and forest plots consistently depicted the correlation between SGLT2 inhibition and insomnia (Fig. 2C, Supplementary Fig. 1A). No significant horizontal pleiotropy was found (Egger intercept = -0.001,  $P = 0.757$ ; global test  $P$ -value = 0.925). No significant heterogeneity was observed, as indicated by the  $P$ -values of 0.902 and 0.830 for the Q statistic in the IVW and MR Egger analysis, and the  $I^2$  statistic was less than 50 % in both methods. The data is displayed in Supplementary Table 3. The leave-one-out analysis result indicates that the effect of SGLT2 inhibition on insomnia is not significantly influenced by any single SNP (Supplementary Fig. 1C).

We also evaluated a causal correlation between SGLT2 inhibition and sleep duration. Using the IVW method, the SGLT2 inhibition was positively associated with sleep duration  $\beta = 0.186$  (95%CI [0.059, 0.314],  $P = 0.004$ ) (Fig. 2B, Supplementary Table 3). Other statistical methods consistently demonstrated a causal relationship between SGLT2 inhibition and sleep duration, thus corroborating the robustness of our main findings. The scatter plot and forest plot illustrate the causal effects of SGLT2 on sleep duration (Fig. 2D, Supplementary Fig. 1B). No apparent horizontal pleiotropy was found (Egger intercept = 0.002,  $P = 0.671$ ; global test  $P$ -value = 0.775). No significant heterogeneity was observed, as indicated by the  $P$ -values of 0.779 and 0.685 for the Q statistic in the IVW and MR Egger analysis, respectively and the  $I^2$  statistic was less than 50 % in both methods. The data is displayed in Supplementary Table 3. The leave-one-out analysis result indicates that the effect of SGLT2 inhibition on sleep duration is not significantly influenced by any single SNP (Supplementary Fig. 1D).

### 3.2. Two-step MR of SGLT2 inhibition, circulating proteins and insomnia

#### 3.2.1. SGLT2 inhibition to circulating proteins (step1 MR)

A two-sample MR study was conducted, using SGLT2 inhibition as the exposure and 4907 plasma proteins as the outcome. Following FDR correction, the IVW method was employed and revealed significant effects of SGLT2 inhibition on 593 plasma proteins (Fig. 3A and B,



**Fig. 2.** The causal estimates between SGLT2 inhibition on insomnia

A and B. The forest plots to visualize the causal effects of SGLT2 inhibition on insomnia and sleep duration. IVW, inverse-variance-weighted method; OR, odds ratio; CI, confidence interval; SNP, single-nucleotide polymorphism. C and D. The Scatter plots to estimate the causal effects of SGLT2 inhibition on insomnia and sleep duration. The slope of each line represents the causal relationship of each method.

Supplementary Tables 4 and 5). Additionally, we evaluated horizontal pleiotropy and heterogeneity to assess the stability of the MR findings. None of the 593 proteins showed significant horizontal pleiotropy (all Egger intercept P-values >0.05, global test P-values >0.05). There was also no significant heterogeneity observed (P-value of Q statistic >0.05;  $I^2 < 50\%$ ) within the identified proteins (Supplementary Table 5).

### 3.2.2. "SGLT2 inhibition"-driven proteins to insomnia (step 2 MR)

In our study, we conducted a subsequent two-sample MR analysis using pQTLs of the 593 plasma proteins as the exposure and insomnia as the outcome. The primary method employed for this analysis was the IVW method. Among the 593 plasma proteins, we screened out Ap4A that affected insomnia, using the FDR-corrected threshold of  $P < 0.05$  (Fig. 3C and D, Supplementary Table 6).

### 3.2.3. Analysis for SGLT2 inhibition to Ap4A (step 1 MR)

A two-sample MR study was carried out to investigate the association between SGLT2 inhibition and Ap4A. The MR analysis showed that SGLT2 inhibition was negatively associated with Ap4A ( $\beta = -0.938$ , 95%CI [-1.529, -0.347],  $P = 0.002$ ) using the IVW method. Other statistical models displayed similar estimate directions for the SGLT2 inhibition on Ap4A (Fig. 4A, 4C, Supplementary Table 7). The forest plot of the relationship between SGLT2 inhibition and Ap4A is illustrated in Supplementary Fig. 2A.

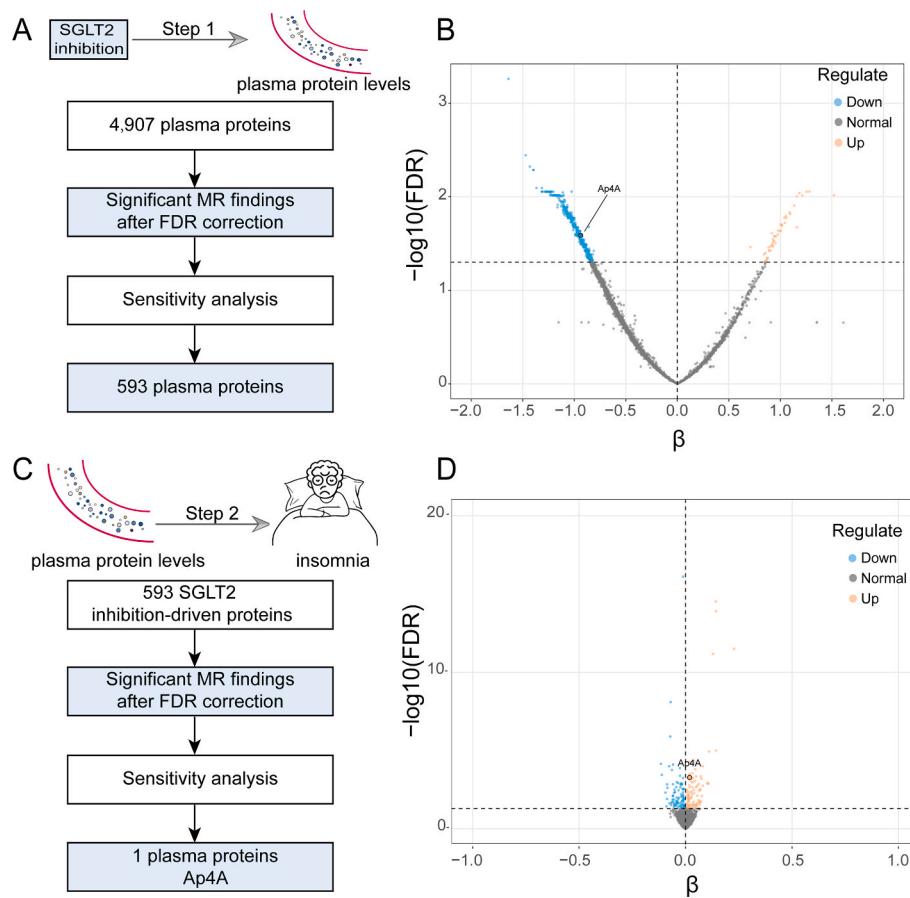
No apparent horizontal pleiotropy was found (Egger intercept =

-0.006,  $P = 0.732$ ; global test P-value = 0.987). No significant heterogeneity was observed, as indicated by the P-values of 0.985 and 0.974 for the Q statistic in the IVW and MR Egger analysis, respectively and the  $I^2$  statistic was less than 50 % in SGLT2 inhibition on plasma Ap4A level (Supplementary Table 7). The Leave-one-out analysis result indicated that the effect of SGLT2 inhibition on Ap4A is not significantly influenced by any single SNP (Supplementary Fig. 2C).

### 3.2.4. Analysis for Ap4A to insomnia (step 2 MR)

In Step 2 MR, we utilized pQTLs associated with Ap4A as the exposure and considered insomnia as the outcome. We identified three SNPs significantly associated with Ap4A, employing a threshold of  $P < 5 \times 10^{-8}$ , and excluded SNPs with potential linkage disequilibrium ( $R^2 > 0.001$ , clump distance <10,000 kb) (Supplementary Table 8). MR results revealed that a one SD increase in genetically predicted AP4A levels was associated with decreased OR of insomnia (OR = 1.019, 95%CI [1.008, 1.031],  $P = 5.2 \times 10^{-4}$ ) of the IVW method. Other statistical approaches displayed consistent directions (Fig. 4B, Supplementary Table 9). The scatter plot and forest plot of the correlation between AP4A and insomnia are illustrated in Fig. 4D, Supplementary Fig. 2B.

In the sensitivity analysis, no significant evidence of pleiotropy was found (Egger intercept = 0.002,  $P = 0.557$ ) involving Ap4A and insomnia. In IVW and MR Egger analysis, the P value of Q statistic was 0.634 and 0.644 respectively, and the  $I^2 < 50\%$  in both methods, indicating there was no apparent heterogeneity in evaluating the impact of



**Fig. 3.** A Two-step MR analysis

A. Flow diagram of step 1 MR analysis. B. Volcano plot illustrating the effect of SGLT2 inhibition on each circulating protein from the MR analysis using the inverse-variance-weighted method. C. Flow diagram of step 2 MR analysis. D. Volcano plot illustrating the effect of SGLT2 inhibition-driven proteins on insomnia from the MR analysis using the IVW method.

plasma Ap4A level on insomnia (Supplementary Table 9). The leave-one-out analysis result indicated that the effect of Ap4A levels on insomnia is not significantly altered by any single SNP (Supplementary Fig. 2D).

### 3.3. Mediation MR analysis linking SGLT2 inhibition with insomnia via Ap4A

Using a two-step MR approach with the “product of coefficient” method, we investigated the mediation of plasma Ap4A levels between SGLT2 inhibition and insomnia. The proportion of mediation effects was calculated by dividing the estimated effect size of Ap4A-mediated effects by the total effect size of SGLT2 inhibition on insomnia. The results demonstrated that plasma Ap4A levels played a partial mediating role in the improvement of insomnia by SGLT2 inhibitor treatment (proportion mediated = 7.7 %, 95%CI [2.1 %, 15.4 %],  $P = 0.023$ ) (Table 1).

“Total effect” indicates the effect of SGLT2 inhibition on insomnia, “direct effect A” indicates the effect of SGLT2 inhibition on Ap4A, ‘direct effect B’ indicates the effect of Ap4A on insomnia and “mediation effect” indicates the effect of SGLT2 inhibition on insomnia through Ap4A. Total effect, direct effect A and direct effect B were derived by IVW; mediation effect was derived by using the delta method. All statistical tests were two-sided.  $P < 0.05$  was considered significant.

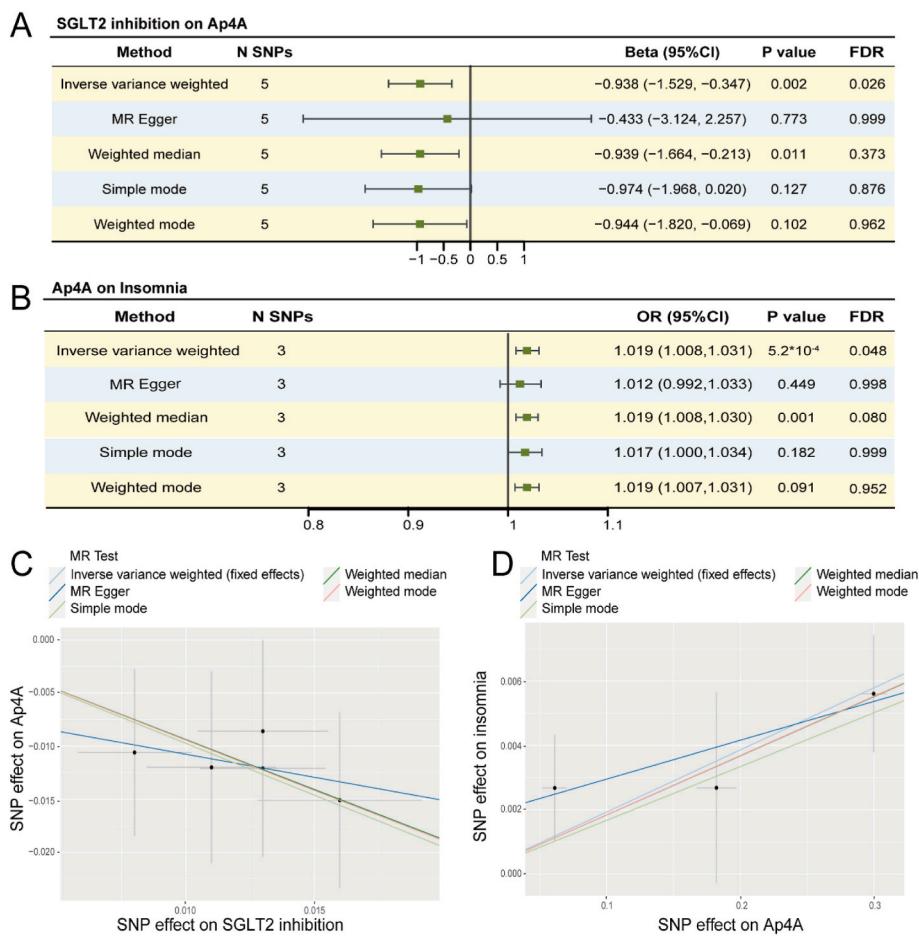
## 4. Discussion

In this study, a two-sample MR analysis showed that SGLT2 inhibition appeared to confer a protective effect against insomnia. Employing

a two-step MR and mediation MR analysis, we identified Ap4A among 4907 plasma proteins, implying that the level of plasma Ap4A might mediate 7.7 % of the effect of SGLT2 inhibition on insomnia. Our data showed that SGLT2 inhibition negatively correlated with circulating Ap4A levels, and plasma Ap4A levels were positively correlated with insomnia.

We sleep almost one-third of our lives. Adequate and regular high-quality sleep is crucial for promoting overall health and well-being [37]. However, sleep disorders, especially insomnia, have become more prevalent and now affect a large portion of people. Meanwhile, insomnia is often dismissed as a minor problem by clinicians. Emerging evidence indicates that sleep can have a significant impact on glycemic status [38]. Hashemipour et al. recently demonstrated that individuals with an evening chronotype, characterized by insomnia and shorter sleep duration, tend to have worse glycemic control [39]. Furthermore, a considerable body of evidence shows associations between insomnia and the risk of stroke [40], obesity [41], type 2 diabetes [42], cognitive impairment [43] and mental abnormalities [44]. Therefore, assessing sleep quality and related parameters should gradually become an integral part of our daily clinical routine, and future studies should pay attention to sleep interventions.

Insomnia is a multifaceted symptom encompassing both primary and secondary insomnia with clinical indications of increased sleep latency and decreased sleep time. Insomnia can also be categorized as initial, middle, late and mixed insomnia based on sleep characteristics. In addition, the most commonly used complaint-based subtype classification focuses on difficulty initiating sleep (DIS), difficulty maintaining sleep (DMS) and early morning awakening (EMA) [45]. The mechanisms



**Fig. 4.** The causal estimates between SGLT2 inhibition on Ap4A and Ap4A on insomnia

A. The forest plot to estimate the causal effect of SGLT2 inhibition on Ap4A. B. The forest plot to visualize the causal effect of Ap4A on insomnia. IVW, inverse-variance-weighted method; OR, odds ratio; CI, confidence interval; SNP, single-nucleotide polymorphism. C. Scatter plot of the causal relationship between SGLT2 inhibition on Ap4A. D. Scatter plot of the causal relationship between Ap4A on insomnia. The slope of each line represents the causal relationship of each method.

**Table 1**  
The mediation effect of SGLT2 inhibition on insomnia through Ap4A.

Mediator	Total effect	Direct effect A	Direct effect B	Mediation effect	p	Mediated proportion (%)
	$\beta$ (95% CI)	$\beta$ (95% CI)	$\beta$ (95% CI)	$\beta$ (95%CI)		(95%CI)
Ap4A	-0.23 (-0.34, -0.13)	0.94 (-1.53, -0.35)	-0.02 (0.01, 0.03)	-0.02 (-0.04, -0.01)	0.02	7.7 (2.1, 15.4)

of insomnia involve various aspects, including physiological, psychological, environmental factors and others. Sleep is under the regulation of circadian rhythms and homeostasis systems. A common cause of primary insomnia is the abnormality in neurotransmitters, including norepinephrine (NE), acetylcholine (Ach), dopamine (DA), glutamate (Glu), 5-hydroxytryptamine (5-HT), Y-aminobutyric acid (GABA) and serotonin [46]. In addition, abnormal expression of sleep-related genes may lead to disruptions in the circadian rhythm; the clock genes include Clock, Cry 1, Cry2, Rora, Bhlhe41, Csnk1d, Per1, Per2, Per3 and others such as Hspa1. Wang et al. have reported that upregulating estrogen receptor 1 (ESR1) and estrogen receptor 2 (ESR2) expression could enhance the contents of 5-HT, GABA, and decreased the contents of Glu, NE, and DA, ultimately regulating sleep-wake disorder [47]. Hence, ESR1 and ESR2 may be possible targets for female sleep-wake regulation

via neurotransmitter regulation. In addition, studies have shown that gut microbiota can regulate host sleep through the brain-gut-bacteria axis [48,49]. What's more, chronic insomnia is an inflammatory-related disease, Aghelan et al. found that NLRP3/caspase-1/IL-1 $\beta$  may serve as a possible therapeutic target for controlling inflammation and alleviating symptoms in chronic insomnia [50].

Ap4A, a dinucleotide metabolite, comprises two adenosines linked in a 5'-5' arrangement by four phosphates [51]. Ap4A is the most prevalent dinucleoside polyphosphate in eukaryotic cells and can be integrated into RNA as a non-canonical initiating nucleotide (NCIN) by RNA polymerases [52]. Using chemical proteomic profiling, Krüger et al. discovered that only around half of these proteins are known nucleotide-binding proteins, indicating that Ap4A's activities in cellular processes extend beyond RNA-associated processes [53]. Although the relationship and mechanism between Ap4A and insomnia have not been reported, the biological processes in which Ap4A is involved may be linked to the regulatory mechanism of insomnia. Miras et al. reported that Ap4A is stored in synaptic secretory vesicles and released upon nerve terminal depolarization, where it triggers presynaptic dinucleotide receptors and subsequent neurotransmitter release. Ap5A, one of the members of the dinucleotide family, has been confirmed to promote glutamate, GABA or acetylcholine release in rat midbrain synaptosomes [54,55]. Guerra et al. identified Ap4A as a pharmaceutical target for STING signaling regulation for the treatment of inflammatory disorders [56]. In conclusion, we can suppose that Ap4A may affect insomnia by

regulating the release of neurotransmitters or by modulating the inflammatory response, but the exact mechanism needs to be further verified.

Several limitations require acknowledgment. First, the proxy of the instrumental variable of SGLT2 inhibition is based on the targeted reduction of HbA1c levels and SLC5A2 gene expression levels rather than the direct effects of SGLT2 inhibitors, which may differ from the actual mechanism of SGLT2 inhibition. In addition, the type, dose, frequency and duration of SGLT2 inhibitors may affect their effects on insomnia. Therefore, these aspects should be considered while interpreting our MR findings. Second, our MR studies were limited to people of European origin to reduce potential biases caused by population stratification and different genetic connections among ancestries. The causal effects in non-European populations warrant further exploration. Third, despite doing numerous sensitivity analyses and employing stringent criteria, as is standard in all MR research, dealing with pleiotropy is still hard to avoid. Lastly, due to the lack of GWAS data on different subtypes of insomnia, we could not analyze the causal effects of SGLT2 inhibition on specific subtypes of insomnia. Whether the findings of this study apply to specific subtypes of insomnia needs more data to support it. Despite these limitations, we found that SGLT2 inhibitor appeared to confer a protective effect against insomnia which expanded the scope of application of SGLT2i and it indicates that patients with diabetes and insomnia may have greater clinical benefit.

## 5. Conclusion

In conclusion, we used a two-step MR, sensitivity tests, and mediation analysis to identify Ap4A as an essential mediator in the effect of SGLT2 inhibition on insomnia. This study sheds new light on how SGLT2 inhibition improves insomnia and gives a new angle for researching insomnia treatments.

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## Data availability statement

The data utilized in this study can be found in the public database. For additional details, please contact the corresponding authors.

## CRediT authorship contribution statement

**Jinlan Luo:** Writing – original draft, Methodology, Formal analysis, Data curation. **Ling Tu:** Writing – review & editing, Validation, Funding acquisition, Conceptualization. **Chenchen Zhou:** Validation, Methodology, Data curation. **Gen Li:** Writing – original draft, Formal analysis, Data curation. **Lili Shi:** Writing – review & editing, Supervision, Conceptualization. **Shuiqing Hu:** Writing – review & editing, Software, Formal analysis, Conceptualization.

## Declaration of competing interest

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

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.sleep.2024.05.036>.

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