Sleep Disorders and Biliary Tact Disease: A Mendelian Randomization

maolan tian
The First Affiliated Hospital of Harbin Medical University

haoran ding
The First Affiliated Hospital of Harbin Medical University

guanlin li
The First Affiliated Hospital of Harbin Medical University

shixin lu
The First Affiliated Hospital of Harbin Medical University

Xianzhi Meng (✉ mengxianzhi@hrbmu.edu.cn)
The First Affiliated Hospital of Harbin Medical University

Research Article

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Abstract

**Background:** Sleep disorders can cause a wide variety of diseases, however, his relationship with cholelithiasis has not been proven. The importance of this study is further indicated by the fact that the population of insomnia is on the rise every year.

**Aims:** By understanding the causal relationship between sleep disorders and cholelithiasis through a Mendelian randomization method, we can better guide human work and rest to improve the quality of human life.

**Methods:** We use exposure factors from UK Biobank and FinnGen consortia as well as outcome variables using Multivariate Univariate as well as Multivariate Multifactor Mendelian randomization methods to analyze the causal relationship between insomnia and cholelithiasis.

**Results:** The results show that Nap during the day can increase the risk of cholelith (OR = 1.55 (1.10, 2.18), P = 0.011, P FDR = 0.078) and broad cholelith (OR = 1.54 (1.09, 2.17), P = 0.012, P FDR = 0.085). And, after further tests of multi-factor Mendelian randomization, the same results were still obtained (Cholelith P =0.04, Broad Cholelith P=0.04).

**Conclusions:** Nap during the day can increase the risk of gallstone disease. This result was obtained by Mendelian randomization, and the present study was proved to be solid by sensitivity analysis.

Introduction

Gallstone disease (gallbladder stones, cholelith) afflicts about 10–20% of adults worldwide and is one of the hepatobiliary diseases causing the highest proportion of socioeconomic burden (Chen et al., 2022). The prevalence of gallbladder stones is increasing, and this is particularly evident in developed countries (O'Neil & Kaplan, 2019). In Germany, more than 175,000 people undergo cholecystectomy each year because of gallstone disease (Gutt et al., 2018). Gallstone risk factors include obesity, T2DM, smoking (Chen et al., 2022), high fasting insulin (Di Ciaula, Wang, & Portincasa, 2019; Ruhl & Everhart, 2000) and NAFLD (Arrese, Cortés, Barrera, & Nervi, 2018). Gallstones can be divided into cholesterol stones (due to disturbances in biliary cholesterol homeostasis) and pigment stones (due to abnormal bilirubin metabolism). Of these, cholesterol stones account for the majority (Chen et al., 2022). Although the mortality rate of cholelithiasis is low, complications (such as cholecystitis, cholangitis, and pancreatitis) are severe, and recurrence of gallbladder stones.

Sleep is a basic biological need for humans, it is important for maintaining human immune function (Besedovsky, Lange, & Haack, 2019), having good sleep quality maintains human mental health (Scott, Webb, Martyn-St James, Rowse, & Weich, 2021), and, sleep itself plays a major role in promoting metabolism (Anafi, Kayser, & Raizen, 2019). Sleep disorders increase the risk of physical and mental disease incidence and have multiple health effects (Van Someren et al., 2015). Although there appear to be important gender differences, poorer sleep quality causes lower SPPB scores, which can
make women's grip strength worse (Denison et al., 2021), and, chronic sleep deprivation weakens our body's defense system, thus making us more susceptible to colds or any other infections (Besedovsky et al., 2019). In addition, sleep plays an important role in the cognitive formation and sleep disorders can impair cognitive abilities in children, adolescents, and adults (Dewald, Meijer, Oort, Kerkhof, & Bögels, 2010; Fortier-Brochu, Beaulieu-Bonneau, Ivers, & Morin, 2012). However, the causal relationship between sleep status and cholelithiasis remains unexplored, and in this article, we will examine seven different sleep disorders: Morning evening person (chronotype), Snoring, Sleeplessness insomnia, narcolepsy, Getting up in the morning, Sleep duration and Nap during day Mendelian randomization was used to unfold the analysis to obtain a causal relationship between the different sleep disorders and gallstone disease.

Randomized controlled trials are considered the gold standard for evaluating medical causality because randomization prevents exposures from being confounded by known and unknown confounders (Stel, Jager, Zoccali, Wanner, & Dekker, 2007). However, many clinical trials may not be able to do randomized controlled trials. In contrast, Mendelian randomization studies exploit the random distribution of genetic variants during meiosis to achieve effects similar to those of random assignment in RCT studies (Skrivankova et al., 2021). Mendelian randomization has three main assumptions: a. Genetic variation is associated with risk factors (correlation); b. Genetic variation is independent of confounders (independence); and c. Genetic variation affects outcomes only through risk factors (exclusivity) (Skrivankova et al., 2021). The Inverse Variance Weighting (IVW) method is the most commonly used in Mendelian randomization and is used when all genetic instruments are valid or when the horizontal multiplicity of all variants is zero. Egger regression methods assume that the overall effect of horizontal pleiotropy, if present, is not related to exposure factors (Tin & Köttgen, 2021). Mendelian randomization studies may be biased if the genetic variation is pleiotropic (Emdin, Khera, & Kathiresan, 2017). In addition, tests for heterogeneity should be performed (Emdin et al., 2017). For screening out heterogeneity in SNPs, we can calculate Cochran's Q statistic when there is true heterogeneity, especially when there are significant outliers or when evidence of a causal effect depends on one or a small number of variants, the reliability of causal conclusions is highly questionable (Burgess et al., 2019). MR-Presso has three components including a) horizontal pleiotropy detection (global test); b) horizontal pleiotropy correction by removing outliers (outlier test); and c) testing for differences in causality before and after correction for outliers (distortion test) (Verbanck, Chen, Neale, & Do, 2018). We can use MR-Presso to detect the effect of a single SNP on causality and remove it. Leave-one-out analyses can also identify outliers that significantly affect the causal effect, and removing these data, reduce the effect on the experimental results (Burgess et al., 2019).

**Material And Method**

**Study Design**

In this study, we carried out a two-sample Mendelian randomization (MR) study using data based on publicly available summary-level data from large genome-wide association studies (GWAS) or consortia.
Single Nucleotide Polymorphisms (SNP) were used as an instrumental variable for analysis. Effective MR results depend on the satisfaction of three prerequisite assumptions:

First, instrument variables should be strongly correlated with exposure.

Secondly, instrument variables cannot be associated with any confounders.

Thirdly, instrumental variables should merely influence the risk of outcomes through risk factors and not through alternative means. (Skrivankova et al., 2021)

**SNP Selection**

Firstly, SNPs had a genome-wide significant association \( (P < 5\times 10^{-8}) \), and minor allele frequency (MAF) > 0.01 were selected as candidate SNPs.

Secondly, SNPs in Linkage disequilibrium (LD) \( (r^2 > 0.01 \text{ and clump window } < 10 \text{ kb}) \) were excluded based on the 1000 Genomes LD reference panel (European population), and the SNP with the lowest \( P \) value was retained.

Thirdly, SNPs that were not present in the outcome data were discarded, and SNPs with a strong association with the outcome \( (P < 5\times 10^{-8}) \) were also excluded from the analysis.

Fourthly, palindromic SNPs with intermediate allele frequencies were excluded from the analysis.

Fifthly, Candidate IVs are queried in the PhoneScan database, and IVs associated with risk factors that are associated with the outcome would be excluded.

We used the statistical index “F-value” to quantify the correlation between each SNP and exposure. In general, an F-value greater than 10 indicates a strong correlation between SNP and exposure, which can effectively avoid bias due to weak instrumental variables. The \( f \)-value is calculated as follows:

\[
F = R^2 *(N-k-1)/ k *(1-R^2).
\]

Here \( N \) represents the number of samples exposed to the GWAS study, \( k \) represents the number of IVs, and \( R^2 \) is the magnitude of IV-explained exposure. The \( R^2 \)-value is calculated as follows:

\[
R^2 = 2*EAF*(1-EAF)^2
\]

**Data Sources**

GWAS data for sleep traits were obtained from the UK Biobank consortia (https://biobank.ctsu.ox.ac.uk). We selected a total of seven sleep traits as exposure factors, including 'sleep duration', 'Getting up in the morning', 'chronotype', 'Nap during the day', 'insomnia', 'Snoring', and 'narcolepsy'.
GWAS data for the biliary disease were obtained from the FinnGen consortia (https://www.nngen.fi/). Seven biliary diseases, including Malignant neoplasm of the biliary tract, Cholelithiasis, Cholecystitis, Cholelithiasis-broad definition with cholecystitis, Other diseases of the gallbladder, primary sclerosing Cholangitis (PSC), PSC with reimbursement, were available for analysis.

Two-sample Mendelian randomization

Random effect inverse variance weighted approach was employed as the primary MR analysis since it could avoid the influence of confounding factors in the absence of a horizontal pleiotropic effect.

The weighted median method, MR-Egger regression, and MR-PRESSO (Pleiotropy Residual Sum and Outlier) model were performed to assess the robustness of the results. The IVW, Weighted median, and MR-Egger methods differ from each other in many ways, but they are also related to each other. Weighted median, which assumes that at least 50% of the SNPs are valid, could yield consistent causal estimates with IVW. The MR-Egger regression could yield consistent causal estimates with IVW, assuming that less than 50% of the SNPs are valid.

Multi-factor analysis

In the process of conducting single-factor analysis, the influence of exposure factors on each other could not be excluded, so we again adopted a multi-factor analysis approach.

Statistical analysis

The MR-PRESSO approach could detect possible outliers, and generate estimates before and after the removal of outliers. Similarly, the leave-one-out sensitivity analysis was also used to detect any single SNP that influenced the results greatly.

P value of MR-Egger intercept regression and MR-PRESSO global test was used to indicate pleiotropy. The asymmetry of funnel plots was also used as an indicator of horizontal pleiotropy.

Cochran's Q statistic was applied to quantify the heterogeneity across the selected genetic instruments.

Benjamin Hochberg procedure (FDR) was performed to correct multiple comparisons in multiple hypotheses, and the threshold of P (FDR) was set to 0.10.

Statistical Tools

All analyses were performed on R 4.1.2 software using the “TwoSampleMR” package (version 0.4.25), MRPRESSO packages (version 1.0) and “phenoscanner” package (version 1.0).

TABLE 1
<table>
<thead>
<tr>
<th>outcome</th>
<th>exposure</th>
<th>method</th>
<th>OR</th>
<th>OR(95%CI)</th>
<th>pval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholelith</td>
<td>Nap during day</td>
<td>IVW</td>
<td>1.56</td>
<td>1.11</td>
<td>2.19</td>
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<td>Nap during day</td>
<td>Weighted median</td>
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<td>1.11</td>
<td>2.70</td>
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<tr>
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<td>MR Egger</td>
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<td>1.09</td>
<td>14.85</td>
</tr>
<tr>
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<td>MVMR</td>
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<td>1.04</td>
<td>4.09</td>
</tr>
<tr>
<td>Cholelith</td>
<td>Nap during day</td>
<td>MR PRESSO Raw</td>
<td>1.56</td>
<td>1.11</td>
<td>2.19</td>
</tr>
<tr>
<td>Broad cholelith</td>
<td>Nap during day</td>
<td>IVW</td>
<td>1.55</td>
<td>1.10</td>
<td>2.18</td>
</tr>
<tr>
<td>Broad cholelith</td>
<td>Nap during day</td>
<td>Weighted median</td>
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<td>1.05</td>
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<tr>
<td>Broad cholelith</td>
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<td>MR Egger</td>
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<td>1.00</td>
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<td>Nap during day</td>
<td>MR PRESSO Raw</td>
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<td>1.10</td>
<td>2.18</td>
</tr>
</tbody>
</table>

**TABLE 2 A**

<table>
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<th>outcome</th>
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<th>OR</th>
<th>OR(95%CI)</th>
<th>pval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholelith</td>
<td>Nap during day</td>
<td>MVMR</td>
<td>2.06</td>
<td>1.04</td>
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<td>Broad cholelith</td>
<td>Nap during day</td>
<td>MVMR</td>
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</tr>
</tbody>
</table>

**B**

<table>
<thead>
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<th>outcome</th>
<th>exposure</th>
<th>method</th>
<th>OR</th>
<th>OR.95L</th>
<th>OR.95H</th>
<th>pval</th>
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<td>Nap during day</td>
<td>MVMR</td>
<td>1.66</td>
<td>1.17</td>
<td>2.36</td>
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</tr>
</tbody>
</table>

Table A indicates the results of multivariate multifactor MR, and Table B indicates the results of lasso regression.

**Result**

**MR analysis**
Among the tested sleep trait, IVW analysis indicated that Nap during the day increased the risk for cholelith (OR = 1.55 (1.10, 2.18), P = 0.011, P FDR = 0.078) and cholelith with broad definition (OR = 1.54 (1.09, 2.17), P = 0.012, P FDR = 0.085). (The calculations between various sleep disorders and cholelithiasis are shown in Supplementary Table S1).

The results from MR methods showed a consistent direction. Figure 1. And Odds ratios (ORs) with corresponding confidence intervals (CIs) for exposure were scaled to the unit listed in Table 1.

Multivariate multi-factor Mendelian randomization and the MVMR method further showed that Nap during the day increased the risk for cholelith, while the positive significance of Nap during the day on the occurrence of cholelithiasis was further verified after screening for variables by lasso regression.(Table 2)

In the scatter plot, single nucleotide polymorphism (SNP)-outcome correlations are compared with SNP (cholelith or cholelith broad) correlations, thus visualizing the causal estimates of each SNP for cholelith or cholelith broad. We can visually see that nap during the day is the most important factor promoting cholelith.

Funnel plots of Nap during the day versus cholelith with causal estimates based on individual genetic variants, where causal effects are expressed as the inverse of the standard error of Nap during the day versus cholelith. The overall causal estimates (beta coefficients) of Nap during the day versus cholelith were estimated by IVW (red line) and MR-Egger method (cyan line).

MR leave-one-out sensitivity analysis for Nap during the day on Cholelith.

Circles indicate the results of MR calculations using the IVW method for the remaining SNPs if this SNP is omitted, which indicates the degree of influence of a single SNP on causality. Bars indicate CI.

**Sensitivity analysis**

MR-Egger intercept regression did not indicate pleiotropy of Nap during the day on gallstone (P = 0.145) and gallstone with broad definition(P = 0.185). The MR-PRESSO test identified no outlier SNP of Nap during the day on the risk of gallstone and gallstone with a broad definition. The funnel plots were plotted and showed symmetric results. (Figure2).

However, heterogeneity was observed in Cochran’s Q test analysis of Nap during the day on gallstone with broad definition (Q = 58.60, P = 0.045), since we used a random effect model IVW method, heterogeneity does not affect the stability of the results. And no heterogeneity was suggested across the selected genetic instruments of Nap during the day on the risk of gallstone (Q = 56.32, P = 0.068).

The leave-one-out analysis did not detect a single SNP that strongly influenced the results, suggesting that the MR analysis results were robust. The forest plot of the leave-one-out analysis was shown in Figure 3.
To reduce the mutual interference between different exposure factors, we conducted a multivariate, multifactorial Mendelian randomization analysis to reduce the reliability of the experimental results due to the mutual interference between different exposure factors. We can see that the p value of nap during the day is still less than 0.05, and the multi-factor Mendelian randomization of multiple samples is still significant, indicating the high reliability of the results of this analysis.

We also performed reverse MR analysis, and the results showed no reverse causality. Detailed information was shown in Supplementary Table S2.

**Statistical validity**

Detailed information on the IVs is provided in Supplementary Table S3, We can see that the range of F values is 29.72175 to 166.638, which is much greater than 10, indicating that the statistical validity of the screened SNPs is strong and is fully compliant with the criteria.

**Discussion**

In this article, we can clearly see that IVW analysis indicated that Nap during the day increased the risk for gallstone (OR = 1.55 (1.10, 2.18), P = 0.011, P FDR = 0.078) and gallstone with broad definition (OR = 1.54 (1.09, 2.17), P = 0.012, P FDR = 0.085). We used Mr-presso to detect no effect on the calculated results after removing the outliers (Supplementary Table S4). At the same time, our examination of horizontal pleiotropy(P = 0.145946 and P = 0.185378) and heterogeneity ((Q = 58.60, P = 0.045)and(Q = 56.32, P = 0.068) ) suggests that the results of this study are solid. We used a multifactorial analysis, which reduced the effect between exposure factors, to obtain p-values that confirmed our experimental results even more. By reading the literature, it can be understood that this result may be related to sleep inertia, i.e., a reduction in the separation of functional brain networks from the default mode network (also observed during sleep and high drowsiness).(Picchioni, Duyn, & Horovitz, 2013)

In this article, we used a two-sample Mendelian randomization study based on a large GWAS sample to explore the relationship between Morning evening person (chronotype), Snoring, Sleeplessness insomnia, narcolepsy, Getting up in Morning, Sleep duration, and Nap during the day found to have a causal relationship with cholelithiasis, and the results showed that nap during the day had a facilitative effect on the development of cholelithiasis. And the sensitivity analysis showed that this finding was robust.

Daytime naps have a positive effect on human health and increase productivity afterwards. The restorative effects of daytime naps on humans have been well recognized and are supported by scientific evidence. But the study by Rajiv Dhanda and Harjyot Sohalb found that long and frequent naps during the day may have adverse long-term health consequences.(Dhand & Sohal, 2006)

While it is well documented that the benefits of daytime naps can last up to 2.5 hours, (Brooks & Lack, 2006) the opposite result is achieved during the inert phase of sleep, i.e., upon awakening.(Tietzel & Lack, 2002) The positive effects of napping exist mainly in the 30 to 120 minutes after waking up.(Dutheil et
al., 2021) After thirty minutes of napping, the effects on health are subtle. The positive effects on health are diminished and may even be reversed, depending on sensitivity analysis and more so with sleep inertia.(Dutheil et al., 2020)

Previous literature has shown that napping during daytime hours has a more pronounced positive impact on health, especially cognitive performance—This may be because naps longer than 30 minutes create sleep inertia, and the benefits of napping only become apparent after a delay.(Brooks & Lack, 2006) Sleep inertia demonstrates the transition from the sleep to the waking state in humans.(Lovato & Lack, 2010) It is characterized by a decrease in the ability to think and execute commands in the waking state due to sleep.(Brooks & Lack, 2006) The more prolonged naps produce a far greater number of slow-wave naps than short naps, and the number of slow-wave naps is proportional to the amount of sleep inertia.(Dutheil et al., 2020) (Muzet, Nicolas, Tassi, Dewasmes, & Bonneau, 1995) Therefore, in order to avoid inert sleep, we should try to keep our nap time at about 3 minutes.

Paradoxically, however, for older adults daytime napping increases the risk of cardiovascular disease, falls and cognitive impairment, (Brassington, King, & Bliwise, 2000; Liu & Liu, 2005; McCrae et al., 2006; Stone et al., 2006) as well as death.(Bursztyn, Ginsberg, Hammerman-Rozenberg, & Stessman, 1999; Bursztyn, Ginsberg, & Stessman, 2002; Bursztyn & Stessman, 2005; Newman et al., 2000) And, as we have felt many times, daytime naps will inevitably reduce the quality of sleep at night.(Faraut, Andrillon, Vecchierini, & Leger, 2017)

These findings can also show from the side that daytime naps are beneficial and detrimental to human health, but there is no clear statement on the specific mechanism that causes gallstone disease to occur until now.

**Limitations**

The present article though shows that daytime napping is associated with an increased prevalence of cholelithiasis and meets the criteria in terms of horizontal pleiotropy as well as heterogeneity, with solid results obtained from sensitivity analysis. However, there is not into a step to show which specific way of daytime napping (e.g., length of sleep, specific time period of sleep occurrence, frequency of slow wave occurrence during sleep,) influences the occurrence of cholelithiasis, and there is no further segmentation of exposure factors, so there is a big lack in this part. Therefore, the next goal of our team is to find clear and meaningful factors influencing cholelithiasis in these subdivisions. Although Mendelian randomization methods have some advantages over traditional meta-analyses in providing evidence for an association between Nap during the day and Cholelith, they are still modeling experimental and hypothesis-dependent.

**Conclusions**
Our findings suggest a causal relationship (positive correlation) between nap during the day and cholelithiasis, but no single mechanism can explain this phenomenon. This certainly provides a new direction for research and a warning for people who need to pay more attention to their lifestyles for daytime napping.

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References


**Figures**
Figure 1

In the scatter plot, single nucleotide polymorphism (SNP)-outcome correlations are compared with SNP (cholelith or cholelith broad) correlations, thus visualizing the causal estimates of each SNP for cholelith or cholelith broad. We can visually see that nap during the day is the most important factor promoting cholelith.
Figure 2

Funnel plots of Nap during the day versus cholelith with causal estimates based on individual genetic variants, where causal effects are expressed as the inverse of the standard error of Nap during the day versus cholelith. The overall causal estimates (beta coefficients) of Nap during the day versus cholelith were estimated by IVW (red line) and MR-Egger method (cyan line).
Figure 3

MR leave-one-out sensitivity analysis for Nap during the day on Cholelith. Circles indicate the results of MR calculations using the IVW method for the remaining SNPs if this SNP is omitted, which indicates the degree of influence of a single SNP on causality. Bars indicate CI.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableS1.xlsx
- SupplementaryTableS2.xlsx
- SupplementaryTableS3.xlsx
- SupplementaryTableS4.docx