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Research Article

Elucidating the Causal Associations Between Disturbances in Sleep-Wake Cycles and Metabolites: A bidirectional Mendelian Randomization Study

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Abstract

Background: The precise role of metabolites in the pathogenesis of sleep-wake cycle disturbances remains unclear. This study introduces bidirectional Mendelian randomization (MR) as a tool for exploring causality and underlying mechanisms.

Methods: Bidirectional MR analysis was conducted using a comprehensive set of 1091 human blood metabolites and 309 metabolite ratios, systematically probing potential causal associations with sleep-wake cycle disturbances. Genome-wide association study (GWAS) data pertaining to these conditions were obtained from the European Bioinformatics Institute and the Finnigen GWAS project. Sensitivity analyses were performed to evaluate heterogeneity and pleiotropy.

Results: Following rigorous genetic variant selection, significant associations were identified based on PIVW < 0.05, PWM < 0.05, and PMR-Egger < 0.05 criteria in the MR analysis. Metabolites significantly associated with sleep-wake disturbances included 1-palmitoyl-GPC (16:0) (PIVW=0.005, odds ratio (ORIVW)=0.79, 95% confidence interval (CI): 0.652–0.956), 1-stearoyl-2-oleoyl-GPI (18:0/18:1) (PIVW=0.005, ORIVW=0.85, 95%CI: 0.732–0.987), N-acetylphenylalanine (PIVW=0.016, ORIVW=1.265, 95%CI: 1.073–1.493), bilirubin (PIVW=0.007, ORIVW=0.992, 95%CI: 0.987–0.998), and octadecanedioylcarnitine (PIVW=0.033, ORIVW=1.006, 95%CI: 1.001–1.012). Conversely, N-methyl-2-pyridone-5-carboxamide (PIVW=0.047, ORIVW=0.389, 95%CI: 0.153–0.988), 2-linoleoylglycerol (PIVW=0.047, ORIVW=0.365, 95%CI: 0.135–0.986), and X-11847 (PIVW=0.049, ORIVW=2.493, 95%CI: 1.002–6.204) were identified as key metabolites significantly affected by disturbances in the sleep-wake cycle. No evidence of reverse causality was found between the sleep-wake cycle disturbances and the metabolites.

Conclusion: This study established causal relationships between several metabolites and sleep-wake cycle disturbances and identified significant biomarkers for screening and prevention. Metabolic disruption plays a crucial role and offers insights for future research and clinical strategies.

Keywords*:* Causality, Mendelian Randomization, Sleep Disturbances

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Introduction

The sleep-wake cycle, a core component of the human circadian system, is essential for maintaining physiological functions, emotional stability, and cognitive performance [1, 2]. Disturbances in this cycle can lead to sleep-wake disturbances, which significantly affect an individual's quality of life and overall health. These disturbances, categorized as delayed sleep-wake phase disorders, advanced sleep-wake phase disorders, non-24-hour sleep-wake rhythm disorders, and irregular sleep-wake rhythm disorders, have become major public health concerns. The disruption of these rhythms not only impairs

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sleep patterns and quality but also adversely affects daytime functioning, causing attention deficits, memory problems, mood swings, and decreased work performance [3, 4]. Over time, these disturbances can exacerbate or contribute to chronic conditions, such as cardiovascular diseases, diabetes, and mental health disturbances, posing significant long-term health risks.

Given the complex nature of sleep-wake cycle disturbances, it is crucial to explore the underlying biological mechanisms that contribute to these disturbances. One promising avenue for such an exploration is the study of metabolites. Metabolites are small molecules that are intermediates and products of metabolism. Their levels can reflect the biochemical activity within cells and tissues. These small molecules are influenced by genetics, diet, lifestyle, and disease states, which significantly affect disease susceptibility and therapeutic targets. Understanding the metabolic alterations associated with sleep-wake cycle disturbances can provide valuable insights into their pathogenesis and potential therapeutic targets [5].

Mendelian randomization (MR) is a novel analytical method that has been extensively used to deduce causal relationships between exposures and outcomes. In contexts where randomized controlled trials (RCTs) are unavailable or where new RCTs are being considered, MR offers a vital alternative, providing robust evidence for the causal links between exposures and disease risks. MR uses single nucleotide polymorphisms (SNPs) as instrumental variables (IVs) that are not confounded to represent the phenotypes under investigation. Due to the random allocation of genetic variants at fertilization, analogous to an RCT, confounding variables, such as sex and age, are less likely to distort the causal inference. Additionally, because genotypes are established before disease onset and remain unaffected by disease progression, the issue of reverse causality is largely mitigated [6, 7].

Given the unclear causal impact of metabolites on sleep-wake cycle disturbances, we employed genome-wide association study (GWAS) summary data within a two-sample MR framework to systematically evaluate potential causal relationships between metabolites and sleep-wake cycle disturbances. To comprehensively identify the candidate metabolites related to the etiology of sleep-wake cycle disturbances, we implemented an exhaustive exposure design encompassing 1400 metabolites. The findings of this study not only enhance our understanding of the pathophysiological mechanisms underlying sleep-wake cycle disturbances but also provide a robust foundation for developing feasible screening and prevention strategies in clinical settings.

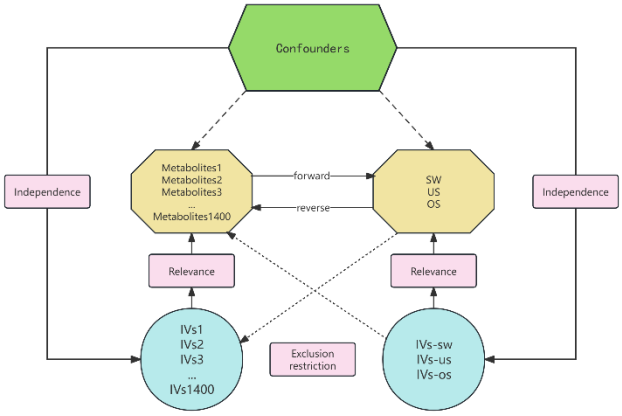
1. Materials and methods

1.1 Study design

We employed a bidirectional two-sample MR approach to investigate the causal relationships between metabolites and sleep-wake cycle disturbances. A rigorous MR analysis involves scrutinizing three pivotal assumptions: (1) genetic IVs must demonstrate a substantial association with the exposure under examination; (2) these IVs should not be associated with any known or unknown confounding factors and must be irrelevant to the outcome except through exposure; and (3) the impact of IVs on the findings should be solely mediated by the exposure under examination [8, 9].

Figure 1 depicts the study design and data sources. Summary-level data were obtained from the GWAS, including 1400 metabolites as genetic IVs [10]. Initially,

the metabolites were treated as exposures, and sleep-wake cycle disturbances were treated as outcomes to elucidate their potential impacts on these disturbances. Subsequently, sleep-wake cycle disturbances were considered as exposures, and metabolites were considered as outcomes to explore post-disease occurrence changes in metabolites. The summary-level data used in this study are publicly available and have received ethical approval from the relevant institutions overseeing each GWAS.



1.2 Data sources

Data on sleep duration among 'undersleepers' were retrieved from the European Bioinformatics Institute (EBI), specifically from the article with PMID 27494321. This investigation focused on the European population, comprising 28,980 cases and 81,208 controls, resulting in a total sample size of 110,188 individuals. Similarly, data on sleep duration among 'oversleepers' were retrieved from the same source. This analysis included 10,102 cases and 81,204 controls from the European population, resulting in a sample size of 91,306 individuals. Additionally, data regarding sleep-wake schedule disorders were acquired from the Finngen GWAS project initiated by the Finnish Genetics Initiative. This large-scale study collected data from the European population comprising 456 cases and 405,229 controls, resulting in a total sample size of 405,685 individuals. For further details, please refer to the Finngen GWAS project (https://storage.googleapis.com/finngen-public-data-r10/summary_stats/finngen_R10_F5_SLEEPWAKE.gz).

We acquired GWAS summary statistics concerning blood metabolites and metabolite ratios from the GWAS Catalog. Specifically, we utilized data from the European GWAS identified as GCST90199621–90201020. The analysis included 8299 unrelated individuals of European descent who participated in The Canadian Longitudinal Study on Aging (CLSA). A comprehensive evaluation was conducted, involving approximately 15.4 million single nucleotide polymorphisms (SNPs) tested for association with 1091 blood metabolites and 309 metabolite ratios [10].

1.3 Selection criteria for genetic variants

For the MR analysis, IVs were chosen based on three fundamental assumptions. Initially, a stringent genome-wide significance threshold of $P < 5 \times 10^{-8}$ was selected, which resulted in an insufficient number of SNPs. Therefore, the threshold was subsequently adjusted to $P < 1 \times 10^{-5}$ to ensure an adequate detection of strongly correlated SNPs. Following this adjustment, to mitigate the influence of linkage disequilibrium, a threshold of $R^2 < 0.001$ was set within a 10,000 kb distance, effectively enhancing the robustness of our analysis. Finally, to ascertain the suitability of the selected SNPs as robust instruments, we employed a threshold of $F > 10$ to ensure the reliability of the IVs. This rigorous selection process guaranteed the validity and reliability of the results obtained from the MR analysis.

1.4 Statistical analysis

Utilizing the "fastMR" package within R software (version 4.3.2), we applied the odds ratio (OR) methodology to gauge the amplitude and trajectory of metabolic influence, accompanied by their respective 95% confidence intervals (CIs). This investigation employed the standard inverse variance weighting (IVW) technique as its primary analytical approach, delving into the causal nexus between metabolites and sleep-wake disturbances encompassing sleep-wakefulness, obstructive sleep apnea, and unspecified sleep disturbances. Both forward and reverse MR analyses were conducted to comprehensively investigate these relationships. Additionally, the MR-Egger and weighted median (WM) methods served as secondary assessment tools. The IVW method provides a more precise estimation of the causal exposure effect when the IVs adhere to the three principal assumptions, establishing it as the most effective MR technique.

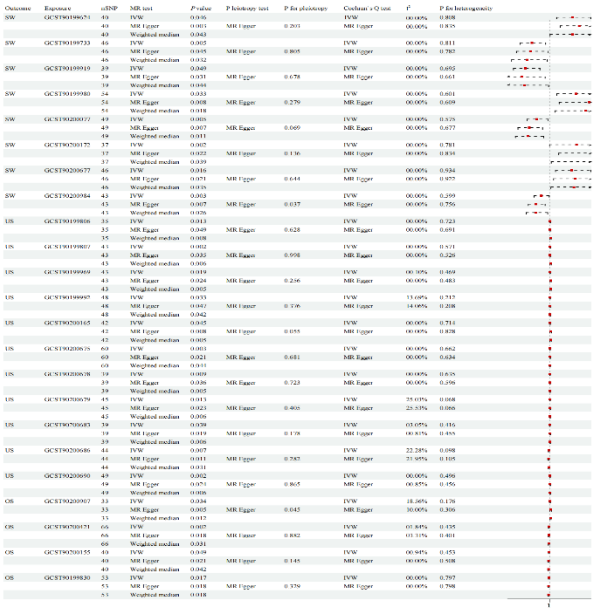
To mitigate false positives, we conducted a rigorous MR analysis, where achieving $P_{IVW} < 0.05$ and $P_{WM} < 0.05$ indicated robust positivity. Furthermore, we employed even stricter thresholds—$P_{IVW} < 0.05$, $P_{WM} < 0.05$, and $P_{MR\text{-}Egger} < 0.05$—to decrease the likelihood of false positives. However, if some IVs fail to meet the IV assumptions, this may lead to inaccurate results. Therefore, we performed the following sensitivity analyses: 1) When the p-value from Cochran's Q test was greater than 0.05 and the I-squared (I^2) statistic was less than 25%, there was no heterogeneity; [11-13] 2) implementation of the MR-Egger intercept method to estimate horizontal pleiotropy, ensuring the independence of genetic variation from metabolites and SW, OS, and US; [14] 3) supplementary analyses, such as WM and weighted mode, enhancing the reliability and stability of hypothesis testing; [15] and 4) individual SNP analysis and leave-one-out testing to evaluate the plausibility of observed individual SNP correlations [16].

1.5 Reverse MR analysis

A reverse MR analysis was conducted to validate the directionality of the causal relationship. In this analysis, sleep-wake cycle disturbances were treated as exposures and metabolites were considered as the outcome. The results indicated that there was no significant reverse causal relationship between sleep-wake cycle disturbances and the metabolites. These findings confirm the primary direction of causality established in our study.

2. Results

Initially, a stringent genome-wide significance threshold of $P < 5 \times 10^{-8}$ was selected, which resulted in an insufficient number of SNPs. Consequently, the threshold was adjusted to $P < 1 \times 10^{-5}$ to ensure the detection of strongly correlated SNPs. All F statistic values exceeded 10, indicating minimal weak instrumental bias. The study included 1091 blood metabolites and 309 metabolite ratios, primarily fatty and amino acids **(Figure 2)**.

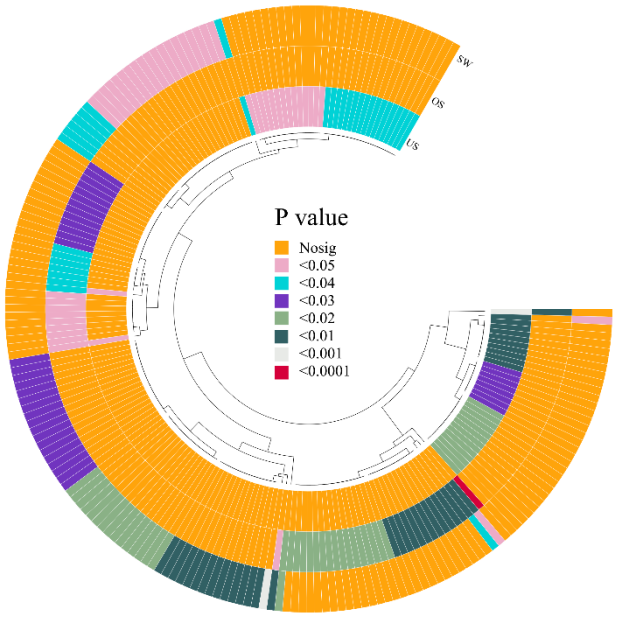


To assess the influence of these 1400 metabolites on sleep-wake cycles, we employed IVW as the primary method, supplemented by MR-Egger and WM approaches. To mitigate false positives, we conducted rigorous MR analysis, with $P_{IVW} < 0.05$ and $P_{WM} < 0.05$ indicating robust associations. Stricter thresholds ($P_{IVW} < 0.05$, $P_{WM} < 0.05$, and $P_{MR\text{-}Egger} < 0.05$) were applied to further reduce the false positives. Sensitivity analyses revealed no heterogeneity when Cochran's Q test p-value was > 0.05, and I^2 was < 25%. Those not meeting these conditions were excluded.

We identified 23 metabolites that were significantly associated with disturbances in the sleep-wake cycle, primarily fatty and amino acids. Notably, GCST90200984 and GCST90200907 exhibited pleiotropy, and GCST90200679 had an I^2 > 25%; therefore, they were excluded. Cochran's Q test revealed no evidence of heterogeneity, and no significant intercept was observed, indicating the absence of pleiotropy. The "leave-one-out" analysis confirmed the robustness of our MR analysis, as the results were not influenced by any individual SNP.

2.1 Metabolite exposure and SW outcome: Robust positive correlation revealed

The identifiers GCST90199624, GCST90199733, GCST90199919, GCST90199980, GCST90200077, GCST90200172, and GCST90200677 corresponded to specific metabolites: hippurate levels, 1-palmitoyl-GPC (16:0) levels, alliin levels, N-formylphenylalanine levels, 1-stearoyl-2-oleoyl-GPI (18:0/18:1) levels, 3-carboxy-4-methyl-5-pentyl-2-furanpropionate (3-CMPFP) levels, and N-acetylphenylalanine levels, respectively. The results of the statistical associations between these metabolites and the outcome variable 'SW' were as follows: hippurate levels (OR_{IVW}=1.273, 95%CI: 1.086–1.492, P_{IVW}=0.046, $P_{MR\text{-}Egger}$=0.003, P_{WM}=0.043), 1-palmitoyl-GPC levels (OR_{IVW}=0.79, 95%CI: 0.652–0.956, P_{IVW}=0.005, $P_{MR\text{-}Egger}$=0.045, P_{WM}=0.032), alliin levels (OR_{IVW}=0.712, 95%CI: 0.571–0.887, P_{IVW}=0.049, $P_{MR\text{-}Egger}$=0.031, P_{WM}=0.044), N-formylphenylalanine levels (OR_{IVW}=1.32, 95%CI: 1.087–1.602, P_{IVW}=0.033, $P_{MR\text{-}Egger}$=0.008, P_{WM}=0.018), 1-stearoyl-2-oleoyl-GPI levels (OR_{IVW}=0.85, 95%CI: 0.732–0.987, P_{IVW}=0.005, $P_{MR\text{-}Egger}$=0.007, P_{WM}=0.011), 3-carboxy-4-methyl-5-pentyl-2-furanpropionate levels (OR_{IVW}=1.325, 95%CI: 1.001–1.754, P_{IVW}=0.002, $P_{MR\text{-}Egger}$=0.022, P_{WM}=0.039), and N-acetylphenylalanine levels (OR_{IVW}=1.265, 95%CI: 1.073–1.493, P_{IVW}=0.016, $P_{MR\text{-}Egger}$=0.021, P_{WM}=0.035). These results suggested a positive association between each of these metabolites and the SW outcome. Moreover, a 'leave-one-out' analysis was conducted to assess the robustness of our MR analysis, which demonstrated its independence from any individual SNP **(Figure 3)**.



2.2 Metabolite exposure and US outcome: Robust positive correlation revealed

The following 10 metabolites, identified by their respective identifiers (GCST90199806, GCST90199807, GCST90199969, GCST90199992, GCST90200165, GCST90200675, GCST90200678, GCST90200679, GCST90200683, GCST90200686, and GCST90200690), exhibited significant associations: tetradecanedioate levels (OR_{IVW}=1.008, 95% CI: 1.002–1.014, P_{IVW}=0.013, $P_{MR\text{-}Egger}$=0.049, P_{WM}=0.008), hexadecanedioate levels (OR_{IVW}=1.009, 95% CI: 1.004–1.015, P_{IVW}=0.002, $P_{MR\text{-}Egger}$r=0.035, P_{WM}=0.006), 1-oleoyl-GPG levels (OR_{IVW}=1.007, 95% CI: 1.001–1.013, P_{IVW}=0.019, $P_{MR\text{-}Egger}$=0.024, P_{WM}=0.005), octadecanedioylcarnitine levels (OR_{IVW}=1.006, 95% CI: 1.001–1.012, P_{IVW}=0.033, $P_{MR\text{-}Egger}$=0.047, P_{WM}=0.042), octadecenedioate levels (OR_{IVW}=1.006, 95% CI: 1.000–1.012, P_{IVW}=0.045, $P_{MR\text{-}Egger}$=0.008, P_{WM}=0.005), N-acetyltyrosine levels (OR_{IVW}=1.006, 95% CI: 1.002–1.011, P_{IVW}=0.003, $P_{MR\text{-}Egger}$=0.021, P_{WM}=0.044), N-acetylasparagine levels (OR_{IVW}=1.005, 95% CI: 1.001–1.010, P_{IVW}=0.009, $P_{MR\text{-}Egger}$=0.036, P_{WM}=0.005), N-acetylcitrulline levels (OR_{IVW}=1.005, 95% CI: 1.000–1.009, P_{IVW}=0.039, $P_{MR\text{-}Egger}$=0.019, P_{WM}=0.006), bilirubin levels (OR_{IVW}=0.992, 95% CI: 0.987–0.998, P_{IVW}=0.007, $P_{MR\text{-}Egger}$=0.011, P_{WM}=0.031), and N-acetyl-1-methylhistidine levels (OR_{IVW}=1.007, 95% CI: 1.003–1.011, P_{IVW}=0.002, $P_{MR\text{-}Egger}$=0.024, P_{WM}=0.006). These metabolites were positively correlated with the 'US' outcome in our study cohort. Additionally, our investigation employed a "leave-one-out" analysis, demonstrating the robustness of the MR methodology utilized. This analytical approach was unaffected by individual SNPs, confirming the reliability of the MR analysis framework.

2.3 Metabolite exposure and OS outcome: Robust positive correlation revealed

The three metabolites, GCST90200421, GCST90200155, and GCST90199830, represented distinct levels of salicylate (OR_{IVW}=0.993, 95%CI: 0.988–0.997, P_{IVW}=0.002, $P_{MR\text{-}Egger}$=0.018, P_{WM}=0.031), 2-furoylcarnitine (OR_{IVW}=0.995, 95%CI: 0.990–1.000, P_{IVW}=0.049, $P_{MR\text{-}Egger}$=0.021, P_{WM}=0.042), and 4-ethylphenylsulfate (OR_{IVW}=0.994, 95%CI: 0.989–0.999, P_{IVW}=0.017, $P_{MR\text{-}Egger}$=0.018, P_{WM}=0.018), respectively. Notably, these three metabolites were positively associated with OS. Our MR analysis demonstrated robustness, as indicated by the "leave-one-out" analysis, which revealed minimal susceptibility to the influence of any individual SNP.

2.4 Influence of sleep-wake cycles on 1400 metabolites

To assess the impact of sleep-wake cycles on 1400 metabolites, we employed the IVW method as our primary approach, complemented by MR-Egger and WM analyses. Initially, associations were sought by screening for P_{IVW} <0.05, P_{WM} <0.05, and $P_{MR\text{-}Egger}$ <0.05, which did not yield eligible results. Subsequently, we refined our screening criteria to include only P_{IVW} <0.05 and P_{WM} <0.05. Three metabolites that were significantly associated with disturbances in sleep-wake cycles were identified.

OS exposure was associated with 1400 metabolites, revealing a robust positive profile. Specifically, the X-11847 levels exhibited a notable association (OR_{IVW}=2.493, 95%CI: 1.002–6.204, P_{IVW}=0.049), as did N-methyl-2-pyridone-5-carboxamide levels (OR_{IVW}=0.389, 95%CI: 0.153–0.988, P_{IVW}=0.047). Both metabolites were positively correlated with OS. Similarly, the US exposure

correlated with 1400 metabolites as outcomes, demonstrating a strong positive profile. Notably, the 2-linoleoylglycerol levels exhibited a significant positive association (OR_{IVW}=0.365, 95%CI: 0.135–0.986, P_{IVW}=0.047) with the US outcome.

3. Discussion

In this study, we employed a bidirectional two-sample MR approach to investigate the causal relationships between metabolites and sleep-wake cycle disturbances. Our findings indicated that 20 metabolites were associated with sleep-wake cycle disturbances. The metabolites included those involved in fatty acid metabolism, amino acid metabolism, bilirubin metabolism, and drug metabolism. Among them, 1-palmitoyl-GPC (16:0), 1-stearoyl-2-oleoyl-GPI (18:0/18:1), 1-oleoyl-GPG, octadecanedioylcarnitine, and octadecenedioate are lipids. N-formylphenylalanine, N-acetylphenylalanine, N-acetyltyrosine, N-acetylasparagine, N-acetylcitrulline, and N-acetyl-1-methylhistidine are amino acids and their derivatives. Terephthalic acid, tetradecanedioate, and hexadecanedioate are organic acids. Bilirubin refers to bile pigments. Alliin and 3-carboxy-4-methyl-5-pentyl-2-furanpropionate (3-CMPFP) are compounds derived from plants. Salicylate, 2-furoylcarnitine, and 4-ethylphenylsulfate are drug and toxin metabolites.

There is a significant association between fatty acid metabolism and disturbances in the sleep-wake cycle. Lipids are the primary energy-storage substances in the body and play a crucial role in energy metabolism. Glucose is the major energy source in the brain during wakefulness. However, during sleep, the brain's energy demands remain high despite a reduced glucose supply. Research has shown that fatty acids and ketone bodies serve as alternative energy sources in the brain during sleep. Brain activity during wakefulness is linked to a high metabolic rate, and sleep, despite the loss of consciousness, incurs substantial energy costs [17]. During sleep, the body's demand for lipid biosynthesis increases, enhancing its capacity for fatty acid transport and binding [18]. This increased capacity ensures that the brain receives an adequate energy supply during sleep. Furthermore, increased fatty acid oxidation activity provides energy and facilitates the removal or recycling of unwanted lipids. This process is vital for maintaining the energy balance and lipid metabolism during sleep. Abnormal fatty acid metabolism leads to the accumulation of fatty acids, resulting in disturbances in fatty acid oxidation. These disturbances disrupt the energy metabolism balance in the body, resulting in reduced energy levels, fatigue, and weakness. This state of fatigue directly impairs sleep quality, leading to disturbances in the sleep-wake cycle [19].

Disturbances in free fatty acid metabolism are thought to play a key role in the pathogenesis of obesity. Under normal conditions, fatty acids undergo complex metabolic processes and are converted into energy. However, in individuals with obesity, fatty acid uptake and absorption capacities are abnormally enhanced, whereas the metabolic rate significantly decreases. This imbalance leads to the storage of excess fatty acids as fats, thereby promoting obesity. Notably, an increased omega-6/omega-3 fatty acid ratio is strongly associated with the risk of obesity. Maintaining a balance between these fatty acids is essential for normal physiological function [20, 21]. Adjusting this ratio is crucial to prevent and control obesity, which is strongly associated with sleep disorders. Obesity is independently and positively associated with the risk of

developing obstructive sleep apnea (OSA). Anatomically, obesity-induced fat accumulation in the throat, a narrowed upper respiratory tract, and increased abdominal fat mass reduce airway patency and increase the risk of OSA. In the lying position, obese individuals experience significantly reduced lung volume, decreased longitudinal tracheal traction, and pharyngeal wall tension, which exacerbate airway stenosis. The effects of OSA on sleep are extensive, affecting night-time sleep quality and causing daytime sleepiness, inattention, decreased cognitive function, and increased risk of accidents [22-24].

Moreover, abnormal fatty acid metabolism can lead to various health issues, including hyperlipidemia and cardiovascular and cerebrovascular diseases. Fatty acid metabolism is a key component in the mechanism of diabetic cardiomyopathy [25]. The intramuscular accumulation of toxic lipid metabolites causes cellular abnormalities that contribute to cardiac remodeling and insufficiency. In the context of overnutrition and obesity, hepatic fatty acid metabolism is often altered, leading to triglyceride accumulation within hepatocytes and resulting in nonalcoholic fatty liver disease. Lipid metabolism plays a critical role in the development of hepatocellular carcinoma. These diseases are typically accompanied by pain and discomfort, such as headaches and dizziness, which interfere with sleep quality. Additionally, patients with health problems caused by abnormal fatty acid metabolism may experience psychological stress and anxiety, leading to sleep issues, such as difficulty falling asleep and frequent awakenings at night. In summary, disruptions in fatty acid metabolism significantly affect the sleep-wake cycle [26-28].

Amino acid metabolism plays a significant role in regulating the sleep-wake cycle. Abnormalities in amino acid metabolism can substantially affect the sleep-wake cycle by influencing the synthesis and release of neurotransmitters and the production of neuroactive metabolites. Amino acids are the building blocks of proteins and serve as precursors for anabolic processes. In particular, abnormal tryptophan metabolism has profound effects on the sleep-wake cycle. Tryptophan is a precursor to serotonin, a key neurotransmitter that is essential for regulating sleep and mood. The disruption of tryptophan metabolism can disturb the sleep-wake cycle, leading to insomnia, decreased sleep quality, and mood swings [29]. Second, metabolic abnormalities in amino acids, such as glutamate and GABA, can affect sleep architecture. Glutamate is the primary excitatory neurotransmitter in the brain, whereas GABA is the major inhibitory neurotransmitter. Imbalances in the metabolism of these amino acids can disrupt excitatory and inhibitory neurotransmission, affecting the stability and continuity of the sleep architecture. This disruption can result in sleep disturbances such as increased light sleep, decreased deep sleep, and diminished sleep quality. The effects of abnormal tyrosine metabolism on sleep are noteworthy. Tyrosine is a non-essential amino acid whose metabolite, norepinephrine, plays a crucial role in maintaining wakefulness. Abnormal tyrosine metabolism can affect norepinephrine levels, making it difficult to achieve deep sleep or causing premature awakenings. This can manifest as decreased sleep quality, sleepiness, and daytime fatigue [30].

Additionally, amino acid metabolism influences the development of atherosclerosis. Atherosclerosis can lead to an insufficient blood supply to the brain, causing hypoxia and affecting sleep quality, potentially leading to insomnia and vivid dreams. Symptoms associated with

atherosclerosis, such as chest tightness, shortness of breath, headache, and dizziness, can also disrupt sleep. Sleep is a critical behavioral factor in the progression of atherosclerosis and cardiovascular events. The interplay between sleep disturbances and atherosclerosis can exacerbate both conditions, leading to sleep-wake cycle disturbances. Finally, abnormalities in amino acid metabolism can disrupt the hydroelectrolytic balance, glucose metabolism, and skin health, resulting in physical discomfort that affects sleep continuity. While no definitive studies have established a direct causal relationship, the existing medical knowledge and clinical observations suggest that these effects are plausible [31, 32].

Bilirubin, a byproduct of hemoglobin degradation, must be metabolized for appropriate excretion. High bilirubin levels are associated with decreased risks of coronary heart disease (CHD) and cardiovascular disease (CVD). Low plasma bilirubin levels, known as hypobilirubinemia, are common in patients with metabolic dysfunction, potentially leading to cardiovascular complications and stroke. Serum bilirubin is a biomarker for many diseases and is often associated with an increased risk of atherosclerosis. There is a bidirectional relationship between sleep and cardiovascular disease risk factors. Cardiovascular problems can negatively affect sleep quality by causing nocturnal dyspnea, angina, or other discomforts that interrupt sleep and affect sleep continuity. Although a direct link between bilirubin metabolism and disordered sleep-wake cycles has not been fully elucidated, abnormal bilirubin metabolism can adversely affect sleep-wake cycles through mechanisms related to cardiovascular diseases [33, 34].

There is a close correlation between drug metabolism and the sleep-wake cycle. Many drugs produce metabolites in the human body that may have pharmacological activities that directly influence the regulatory mechanisms of the sleep-wake cycle. For example, salicylic acid, the active metabolite of aspirin (ASA), has been used as an anti-inflammatory, antipyretic, and analgesic drug for over a century. However, ASA can also lead to excitation of the central nervous system, thereby affecting sleep quality. Additionally, abnormal drug metabolism can result in changes in the concentration and efficacy of drugs in the body, which can subsequently affect sleep. Although there is no direct literature explicitly stating that drug metabolites influence sleep-wake regulatory mechanisms, it is reasonable to speculate that such associations exist based on the general knowledge of drug metabolism and the sleep-wake cycle [35, 36]. Future studies are necessary to further explore this area and provide a more comprehensive understanding of the drug metabolic process in the human body and its potential impact on the sleep-wake cycle.

In this study, three metabolites were significantly associated with the effect of the sleep-wake cycle on 1400 metabolites: X-11847, N-methyl-2-pyridone-5-carboxamide, and 2-linoleoylglycerol. Studies have found significant associations between sleep behavior, particularly sleep duration and renal function, and the risk of cardiovascular disease,Sleep deprivation has also been identified as a key risk factor for impaired metabolic health, which may have a direct negative effect on renal function [37]. Furthermore, N-methyl-2-pyridone-5-carboxamide (2-PY), the main metabolite of nicotinamide, has been identified as a uremic toxin, and its accumulation may lead to various uremic toxic effects. Although direct data on changes in N-methyl-2-pyridone-5-carboxamide levels during the

sleep-wake cycle are limited, the interaction of sleep with many physiological systems suggests that disturbances in the sleep-wake cycle may indirectly affect renal function or related metabolic pathways, thus influencing the levels of N-methyl-2-pyridone-5-carboxamide. Given the close association of sleep with numerous physiological systems, it is plausible to speculate that disturbances in the sleep-wake cycle may lead to an abnormal elevation in N-methyl-2-pyridone-5-carboxamide levels by affecting renal function and related metabolic pathways [38].

Levels of 2-linoleoylglycerol, a diglyceride typically associated with fat and energy metabolism, may be influenced by the overall metabolic state. Disturbances in the sleep-wake cycle can affect individual energy metabolism and appetite regulation, thereby affecting fat intake and metabolism. This can lead to fluctuations in the 2-linoleoylglycerol levels, particularly in patients with chronic sleep deprivation and poor sleep quality. Additionally, 2-linoleoylglycerol is a partial agonist of the human cannabinoid type 1 receptor (CB1), playing a significant regulatory role in the endocannabinoid (eCB) system. eCBs are a group of lipids with regulatory activities in the brain that promote REM sleep by interacting with melanin-concentrating hormone neurons in the lateral hypothalamus. Although the direct relationship between the sleep-wake cycle and 2-linoleoylglycerol levels is not fully established, the key role of the CB1 receptor in sleep regulation and its interaction with 2-linoleoylglycerol suggest that disruptions in the sleep-wake cycle may affect the expression, distribution, or sensitivity of CB1 receptors, indirectly regulating the biological effects and levels of 2-linoleoylglycerol in the body [39, 40].

A major strength of this study was the use of an MR design, which minimized the residual confounding and reverse causality inherent in observational studies, thereby enabling us to investigate the potential causal relationships between metabolites and sleep-wake cycle disturbances. The consistency observed across sensitivity analyses supports the validity of the effect estimates. Additionally, the IVs used in this study were derived from the most recent GWAS data on sleep-related traits, ensuring robust associations with the exposures of interest and enhancing statistical power.

However, this study had several limitations. The GWAS summary statistics on blood metabolites and metabolite ratios were obtained from the GWAS Catalog, which may not have captured the full spectrum of metabolomic variations despite offering a broad range of metabolites, potentially leading to incomplete exposure characterization. Furthermore, the study population comprised individuals of European descent, raising concerns about the generalizability of the findings to other ethnic groups. Different genetic variants may exhibit varying pleiotropic effects across populations, necessitating further studies to validate these findings in diverse cohorts. Future studies should aim to validate these results in other populations and utilize complementary approaches, such as targeted metabolomics, to achieve a more comprehensive metabolic profile. Longitudinal studies are crucial for exploring the causal relationships between metabolites and sleep-wake cycle disturbances. Mechanistic studies are also needed to elucidate how metabolites influence melatonin production, neurotransmitter levels, and the sleep-wake cycle. The development of metabolite-based biomarkers can significantly enhance the prediction and diagnosis of sleep-wake disturbances.

4. Conclusion

This MR study emphasized the causal link between metabolites and sleep-wake cycle disturbances, along with the potential effects of metabolic disruptions on these conditions. In particular, metabolites such as 1-palmitoyl-GPC (16:0), 1-stearoyl-2-oleoyl-GPI (18:0/18:1), 1-oleoyl-GPG, octadecanedioylcarnitine, octadecenedioate, N-formylphenylalanine, N-acetylphenylalanine, N-acetyltyrosine, N-acetylasparagine, N-acetylcitrulline, N-acetyl-1-methylhistidine, terephthalic acid, tetradecanedioate, hexadecanedioate, bilirubin, alliin, 3-carboxy-4-methyl-5-pentyl-2-furanpropionate (3-CMPFP), salicylate, 2-furoylcarnitine, and 4-ethylphenylsulfate were identified as significant biomarkers for screening and preventive clinical practice in managing sleep-wake cycle disturbances. This study also identified key metabolites that were significantly influenced by disturbances in the sleep-wake cycle, including N-methyl-2-pyridone-5-carboxamide, 2-linoleoylglycerol, and X-11847.

These findings underscore the bidirectional relationship between metabolism and the sleep-wake cycle, highlighting the importance of metabolic health in regulating these cycles. By identifying the key metabolites associated with sleep-wake disturbances, our study offers insights into potential diagnostic and therapeutic targets. Future research should validate these results in diverse populations and explore the underlying mechanisms through which these metabolites influence and are influenced by the sleep-wake cycle. This research paves the way for novel approaches for the prediction, diagnosis, and treatment of sleep-wake cycle disturbances, ultimately contributing to improved health outcomes.

Author Contributions

Shanshan Chen and Jinwen Liu conceived and drafted the initial manuscript, playing pivotal roles in structuring the study. Yi Wang, Danhui Wang, Donghui Ling, Yunguang Gao, Deqing Huang, Limei Diao, and Tai Liu contributed to data collection, analysis, and technical support. Jie Zhong and Qianchao He, as corresponding authors, initiated and supervised the project, providing crucial guidance and final approval of the manuscript.

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Declaration of Interest Statement

The authors declare that there are no conflicts of interest.

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