Nature and Science of Sleep

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Causal Relationship Between Micronutrient and Sleep Disorder: A Mendelian Randomization Study

Yingying Jiang[1,*], Siqi Ge[2,*], Chunyang Wang[1], Chen Jin[1], Yumei Zhao[1], Qingying Liu[3]

[1]Department of Neuropharmacology, Beijing Neurosurgical Institute, Capital Medical University, Beijing, People's Republic of China; [2]Department of Neuroepidemiology, Beijing Neurosurgical Institute, Capital Medical University, Beijing, People's Republic of China; [3]Department of Pain Medicine, the First Affiliated Hospital of Zhengzhou University, Henan, 450052, People's Republic of China

*These authors contributed equally to this work

Correspondence: Qingying Liu, Email liuqy1679@163.com

Background: Sleep played an important part in human health, and COVID-19 led to a continuous deterioration of sleep. However, the causal relationship between micronutrient and sleep disorder was not yet fully understood.

Methods: In this research, the genetic causal relationship between micronutrient and sleep disorder was analyzed utilizing a two-sample Mendelian randomization (MR). Single nucleotide polymorphisms (SNPs) were used as instrumental variables. The analyses were conducted using the MR-Egger, inverse variance weighted, weighted mode, weighted median, simple mode, Cochran's Q test and leave-one-out.

Results: Our results suggested that 8 genetically predicted micronutrients participated in sleep disorders, including liver iron (L-iron) and iron in sleeping too much, spleen iron (S-iron) in sleeplessness/insomnia, trouble falling or staying asleep, sleep duration (undersleepers) and nonorganic sleeping disorders, iron metabolism disorder (IMD) and vitamin B12 deficiency anaemia (VB12DA) in narcolepsy, urine sodium (uNa) in narcolepsy, sleep apnea syndrome and sleep disorder, vitamin D (VD) in sleep duration (oversleepers), 25-Hydroxyvitamin D (25(OH)D) in trouble falling or staying asleep.

Conclusion: Our study used Mendelian randomization methods at the SNP level to explore the potential causal relationship among L-iron, iron, S-iron, IMD, uNa, 25(OH)D, VD, VB12DA with certain sleep disorder subtypes. Our results uncovered a micronutrient-based strategy for alleviating sleep disorder symptoms.

Keywords: Mendelian randomization, micronutrient, sleep disorder, causality, vitamin

Introduction

Sleep is a state of natural cycles, reversible dissociation of perception, diminished consciousness, and relative stationarity determined by circadian rhythms and homeostasis,[1] and contributes significantly to the development and retention of emotional, cognitive, and motor functions.[2] Changes in sleep, including in quantity and quality, occurred throughout the lifespan as individuals age.[3] It was estimated that the average incidence of sleep disorders and insomnia symptoms among the common population was 10% (3.9%-22.1%) and 30% (30%-35%), respectively.[4] This range of variation was largely influenced by the tools used to assess sleep.[4] Especially in the wake of the COVID-19 pandemic, the detrimental influences on sleep, particularly insomnia and poor sleep quality, have been well identified.[5] In addition, sleep disorders were also common among pregnant women, and the state of their prenatal sleep could be significantly related to the long-term outcomes for their children, encompassing the risk of developing attention-deficit/hyperactivity disorder (ADHD).[6] Meanwhile, insufficient sleep was a common problem among elderly adults. Research showed that 67.7% of elderly adults reported difficulties in falling asleep or waking up too early.[7]

Received: 23 April 2024
Accepted: 21 August 2024
Published: 26 August 2024

Sleep disorders were known to greatly increase the risk of neurodegenerative diseases (including amyotrophic lateral sclerosis, Huntington's disease, and dementia with Lewy bodies), cardiovascular disease (such as stroke), hypertension, diabetes, depression, anxiety, epilepsy, ADHD, autism spectrum disorder and all-cause mortality.[8–10] Sleep not only had restorative properties, but also played a vital role in energy metabolism, appetite control, and immunity.

The recently released third edition of the International Classification of Sleep Disorders (ICSD-3) classified seven major categories of sleep disorders: insomnia disorders, central disorders of hypersomnolence, sleep-related breathing disorders, circadian rhythm sleep-wake disorders, parasomnias, sleep-related movement disorders, and other sleep disorders.[11]

In studies on the mechanism of sleep disorders, GABAergic neurons in the regions of lateral hypothalamus and ventral tegmental area acted as an arousal signal for wakefulness and limit wakefulness, respectively.[12] Moreover, astrocytes were involved in the modulation of sleep-wake cycle by regulating rapid eye movement (REM) and non-REM sleep.[10] In addition, studies confirmed that macronutrients (amino acids and carbohydrates) were involved in neurotransmitter levels during sleep and influenced sleep patterns.[13] But, the role of micronutrients (such as metal element, mineral and vitamin) in sleep disorders had not received as much attention as macronutrients.

Currently the most extensively studied element when it comes to sleep disorders is iron. For instance, increased brain iron levels were associated with obstructive sleep apnea;[14] Higher serum Zn, Zn/Se, and Zn/Cu were related to reduced self-reported sleep disorders.[15] A recent research revealed that individuals with restless leg syndrome (RLS) have notably elevated calcium, serum copper, selenium, and magnesium concentrations when compared to the control group.[16] But, in another study, serum selenium concentration was independent of the presence and severity of RLS.[17]

Until now, the causal relationship between many micronutrients and sleep disorders has not been definitively concluded. A better method to assess the causal relationship between micronutrient and sleep disorder is Mendelian randomization (MR).[18]

MR, regarded as a naturally occurring randomized trial, assessed the causal relationship between exposure and outcome through genetic variation, effectively limiting environmental confounding and the possibility reverse causality.[19] The independent allocation of alleles during conception implies that the subgroups categorized by these genes should not have systematic differences in confounding factors, thereby emulating a natural experiment that is analogous to a randomized trial.[19] Using single-nucleotide polymorphisms (SNP) as instrumental variables,[20] MR can deduce the causal relationship between micronutrient and sleep disorder. The application of MR to study the potential drug effects of micronutrients that can be targeted by drug intervention and replaced by gene mutations has important clinical significance.

In this research, we implemented genome-wide association studies (GWAS) of micronutrient (liver iron content (L-iron), iron, spleen iron concentration (S-iron), iron metabolism disorder (IMD), urine sodium (uNa), vitamin D (VD), serum 25-Hydroxyvitamin D (25(OH)D), vitamin B12 deficiency anaemia (VB12DA)). Whereafter, our aim was to utilize MR to study the effect of micronutrient on sleep disorder and its subtype to discover potential prevention targets. Hence, we performed a 2-sample MR research to inspect the potential causal effect between micronutrient and sleep disorder.

Methods

Study Design

We made use of 2-sample MR based on the genetic connection from different GWAS for micronutrient (L-iron, iron, S-iron, IMD, uNa, VD, 25(OH)D, VB12DA) (Table 1) and four subtypes of sleep disorder (insomnia, sleep-related breathing disorders, central disorders of hypersomnolence, other sleep disorders). These subtypes contain different phenotypes. As shown in Table 2.

The research design is summarized in Figure 1, which included three critical hypothesis that must be contented in the MR study: 1) the genetic variant (SNP) is closely related to associated exposure; 2) SNP is independent of known or unknown confounders; and 3) SNP affects outcomes only through interest exposure and not through other means.[18] All statistical analysis were applied by the two-sample MR package (version 0.5.6) in R (version 4.2.1).

Table 1 Data for Including Microelement Information in GWAS

Phenotype	No. of SNPs	Case	Control	Sample size	Population	GWAS ID
Liver iron content	9,275,407			32,858	European	ebi-a-GCST90016674
Iron	2,096,457			23,986	European	ieu-a-1049
Spleen iron concentration	9,275,407			35,324	European	ebi-a-GCST90101831
Iron metabolism disorder	16,380,377	146	197,259	197,405	European	finn-b-E4_IRON_MET
Urine sodium	10,783,698			396,020	European	ebi-a-GCST90013989
Vitamin D	4,225,238			418,691	European	ebi-a-GCST90025967
Serum 25-Hydroxyvitamin D	6,896,093			496,946	European	ebi-a-GCST90000618
Vitamin B12 deficiency anaemia	16,380,452	1,707	211,115	212,822	European	finn-b-D3_ANAEMIA_B12_DEF

Table 2 Data for Including Sleep Disorder Information in GWAS

phenotype	Subtype	No. of SNPs	Case	Control	Sample size	Population	GWAS ID
Insomnia	Sleeplessness / insomnia	11,895,062	1,569	0	1,569	Middle Eastern, North African, or Persian	ukb-e-1200_MID
	Trouble falling or staying asleep	9,810,789	1,173	0	1,173	South Asian	ukb-e-20517_CSA
Central disorders of hypersomnolence	Narcolepsy	9,851,867			460,913	European	ukb-b-5776
	Sleeping too much	13,149,723	9,354	36,186	45,540	European	ukb-d-20534
	Sleep duration (oversleepers)	16,563,303	10,102	81,204	91,306	European	ebi-a-GCST006685
	Sleep duration (undersleepers)	16,561,726	28,980	81,208	110,188	European	ebi-a-GCST006686
Sleep-related breathing disorders	Sleep apnea syndrome	24,183,940	13,818	463,035	476,853	European	ebi-a-GCST90018916
Other sleep disorders	Sleep disorders	11,120,383	2,951	358,243	361,194	European	ukb-d-SLEEP
	Nonorganic sleeping disorders	16,380,172	2,214	166,584	168,798	European	finn-b-KRA_PSY_SLEEP_NONORG_EXMORE

Genetic variants associated with micronutrient and sleep disorder

Since there was no uniform and accurate international standard for micronutrient testing, we searched the GWAS database for micronutrients commonly used in clinical practice: iron, zinc, magnesium, calcium, iodine, selenium, copper, fluoride, lead, and vitamins. As mentioned in the Introduction, sleep disorders are classified into seven major categories.[11] We searched for "sleep" in the GWAS database, then conducted RStudio analysis on the available sleep-related databases and the above micronutrients, and finally classified, summarized and presented the positive results according to the types of ICSD-3. Therefore, this paper showed the exposure (micronutrient) and outcome (sleep disorder) that presented positive results after RStudio analysis.

All data for micronutrient[21–25] and sleep disorder[26,27] in initial analysis were obtained from the publicly available Medical Research Council Integrative Epidemiology Unit (IEU) Open GWAS database. With regard to the detailed information of sample population characteristics, data analysis, and gene types could be identified from the original articles.

Briefly, we collected relevant micronutrient data from 8 studies that conducted a GWAS with participants from Europe, including a total of 1,814,050 sample size. Detailed data are summarized in Table 1.

Instrumental variables for the research of sleep disorders were produced by integrating summary statistics from 9 GWAS of 1,717,534 individuals with mainly European and Asian individuals. An overview of GWAS-related information for each sleep disorder is presented in Table 2.

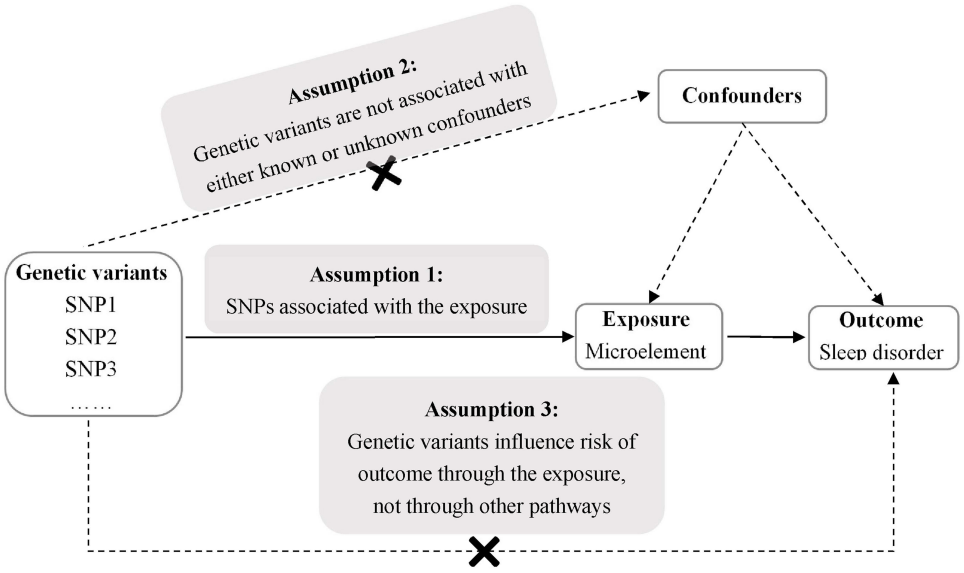

Figure 1 Study design of the two-sample Mendelian randomization for the effect of genetically based connections between micronutrient and subtypes of sleep disorders. SNPs, single-nucleotide polymorphisms.

Selection of instrumental variables

In this MR research, the relationship between micronutrients and sleep disorders was delineated using 2-sample instrumental variable analysis. Initially, we identified SNPs with a significant association ((P-value < 5×10^{-8}) with the exposure by conducting MR analysis. Thereafter, we pinpointed linkage disequilibrium (LD) between the SNPs by applying the "ld_clump" R package to data from the European 1000 Genomes Project. Under the guidelines of pairwise linkage disequilibrium (LD; $r^2 < 0.001$ and clump window > 10,000 kb, among SNPs), SNPs that were independently connected to micronutrient and confounder were identified. The SNPs were displayed as instrumental variables to execute causal relationships. Thirdly, we harmonized the effect alleles of sleep disorder-associated SNPs to correspond with the effect alleles of micronutrient-associated SNPs, aligning by allele letter and allele frequency. A SNP was identified as palindromic on the basis that its minimum LD Rsq value was less than 0.80. The effect alleles for palindromic SNPs were cross-verified with the available information and aligned; however, alleles that could not be indisputably aligned were eliminated.[18]

Statistical Analysis

Employing various types of MR approaches, including inverse variance weighted (IVW), Mendelian randomization Egger regression (MR-Egger), weighted mode, weighted median and simple mode, we aimed to ascertain MR estimates of micronutrient for sleep disorder, following standardized effect allele analysis across GWAS for eight micronutrients and nine sleep disorder subtypes. Utilizing IVW as our primary method for meta-summarization of multi-loci effects, we proceeded under the idealized condition that each SNP was valid and possessed complete independence.[17] Following the extraction of estimates associating variants with micronutrients or sleep disorders, we aligned the direction of the estimates according to the effect alleles. In scenarios with more than one SNP, we employed the IVW method, choosing a fixed-effect model for Cochran's Q test P values exceeding 0.05, or a random-effect meta-analysis for P values less than 0.05.

Furthermore, various MR methods, such as MR-Egger and weighted median, were employed to evaluate the robustness of the study outcomes. The weighted median, calculated as the median of the distribution of individual SNP effect values ranked by weight, allows for less than 50% of SNPs to be invalid, while MR-Egger accommodates potential pleiotropy in all SNPs, assuming that pleiotropic effects on the outcome are independent of the effects on exposure factors. Namely, MR-Egger facilitated the assessment of heterogeneity and pleiotropy across the entire set of

SNPs, even when horizontal heterogeneity is present.[19] When no less than half of the SNPs were valid SNPs, weighted median and weighted model were used to estimate causal effects. Simple mode was conducted to demonstrate whether the observed genotype differences between micronutrients and sleep disorders were statistically significant.

Moreover, we utilized specialized sensitivity analyses to identify and adjust for any underlying heterogeneity and pleiotropy, meeting the essential assumptions of our MR study methodology. To ascertain the heterogeneity of each instrumental variable, we employed the heterogeneity test, commonly referred to as Cochran's Q test.[19] To validate that there is no directional pleiotropy affecting the linkage of micronutrients to sleep disorders, the P-value of Cochran's Q test should be more than 0.05.[19] The choice of IVW model was contingent on the presence of heterogeneity: the fixed effects model for non-significant P values (> 0.05), and the multiplicative random-effects model for significant P values (< 0.05). Moreover, MR-Egger was used to estimate potential biases caused by horizontal pleiotropy and invalid instrumental variables. When the intercept of the regression lacked statistical significance ($P < 0.05$), the slope in MR-Egger regression could act as an estimate of outcome caused by exposure effects in the causal relationship. To enhance the interpretability of our results, we conducted a leave-one-out analysis to identify the influence of individual SNPs associated with exposure, then reiterated the IVW analysis in a sequential process, each time omitting a single SNP. By conducting leave-one-out analysis, the significance of each SNP's impact on the IVW causal estimates was evaluated.[19]

The study outcomes were quantified as OR (odds ratio), complete with 95% confidence interval, and all P values were two-sided with a 5% level set for statistical significance.

Results

GWAS on Micronutrient and Sleep Disorder

Among the micronutrient categories (iron, zinc, magnesium, calcium, iodine, selenium, copper, fluoride, lead, and vitamins) tested by utilizing micronutrient-related SNPs, IVW preliminarily identified 8 micronutrients (L-iron, iron, S-iron, IMD, uNa, VD, 25(OH)D, VB12DA) implying a high-risk on sleep disorder. The GWAS of positive results of 8 micronutrients and 9 sleep disorder subtypes were presented in Table 1, 2. The derived genetic association estimates were subsequently applied in the following MR analyses (Supplementary Figure 1).

Causal effects of the micronutrient on sleep disorder

SNPs were chosen and employed as instrumental variables in the MR analysis to examine the relationship between micronutrients and sleep disorders. The association between 8 micronutrients and 9 sleep disorder subtypes, as determined by MR analyses, is quantified in Supplementary Tables 1 and 2. Cochran's Q test identified heterogeneity in the relationships between 8 factors (L-iron, iron, S-iron, IMD, uNa, VD, 25(OH)D, VB12DA) and certain sleep disorder subtypes. For $P_{(Q)} < 0.05$, we proceeded with the multiplicative random-effect IVW model (Supplementary Table 2), and for variables that did not display heterogeneity ($P_{(Q)} > 0.05$), the fixed-effect IVW model was selected (Supplementary Table 2).

According to the MR-Egger intercept, there is no statistical evidence to suggest directional horizontal pleiotropy for the eight micronutrients and nine sleep disorder subtypes analyzed ($P_{(intercept)} > 0.05$, Supplementary Tables 1 and 2).

In our initial IVW analysis, the results from genetically predicted that liver iron and iron were both negatively associated with sleep too much ($P < 0.05$, $OR < 1$, details as illustrated in Figure 2A and B), providing evidence of a potential causal correlation. The relationship between spleen iron concentration and sleep disorders was complex. Specifically, it was positively related to sleeplessness/insomnia and sleep duration (undersleepers) ($P < 0.05$, $OR > 1$, Figure 2C), and negatively associated with trouble falling or staying asleep and nonorganic sleeping disorders ($P < 0.05$, $OR < 1$, Figure 2C). Iron metabolism disorder was positively related to narcolepsy ($P < 0.05$, $OR > 1$, Figure 2D). The higher the urine sodium, the more severe the narcolepsy, sleep apnea syndrome, and sleep disorder ($P < 0.05$, $OR > 1$, Figure 2E). Moreover, Vitamin D, serum 25-hydroxyvitamin D, and vitamin B12 deficiency anemia were positively correlated to sleep duration (oversleepers), trouble falling asleep or staying asleep, and narcolepsy, respectively ($P < 0.05$, $OR > 1$, Figure 2F-H).

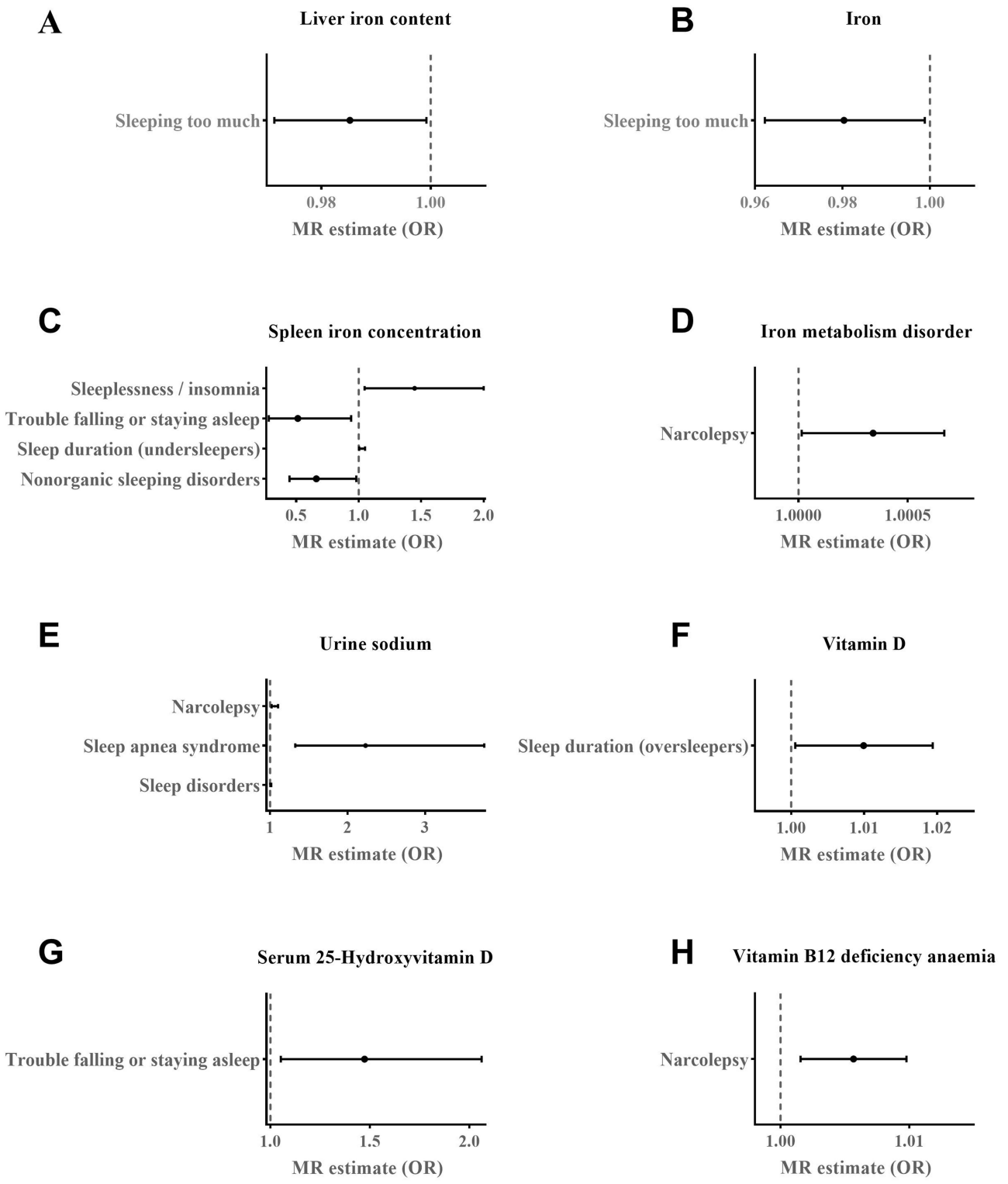

Figure 2 The impact of putatively causal micronutrient on sleep disorder subtypes outcomes. The square was the causal estimates on the OR scale, and the whisker represented the 95% confidence intervals for ORs. OR < 1 indicated a negative correlation between micronutrient and sleep disorder risk, while OR > 1 indicated a positive correlation. (**A** and **B**) Liver iron (**A**) and iron (**B**) were both negative with sleeping too much. (**C**) Spleen iron was positive with sleeplessness/insomnia, sleep duration (undersleepers), and negative with trouble falling or staying asleep, nonorganic sleeping disorders. (**D**) Iron metabolism disorder was positive with narcolepsy. (**E**) Urine sodium was positive with narcolepsy, sleep apnea syndrome and sleep disorders. (**F**) Vitamin D was positive with sleep duration (oversleepers). (**G**) Serum 25-Hydroxyvitamin D was positive with trouble falling or staying asleep. (**H**) Vitamin B12 deficiency anaemia was positive with narcolepsy. OR, odds ratio.

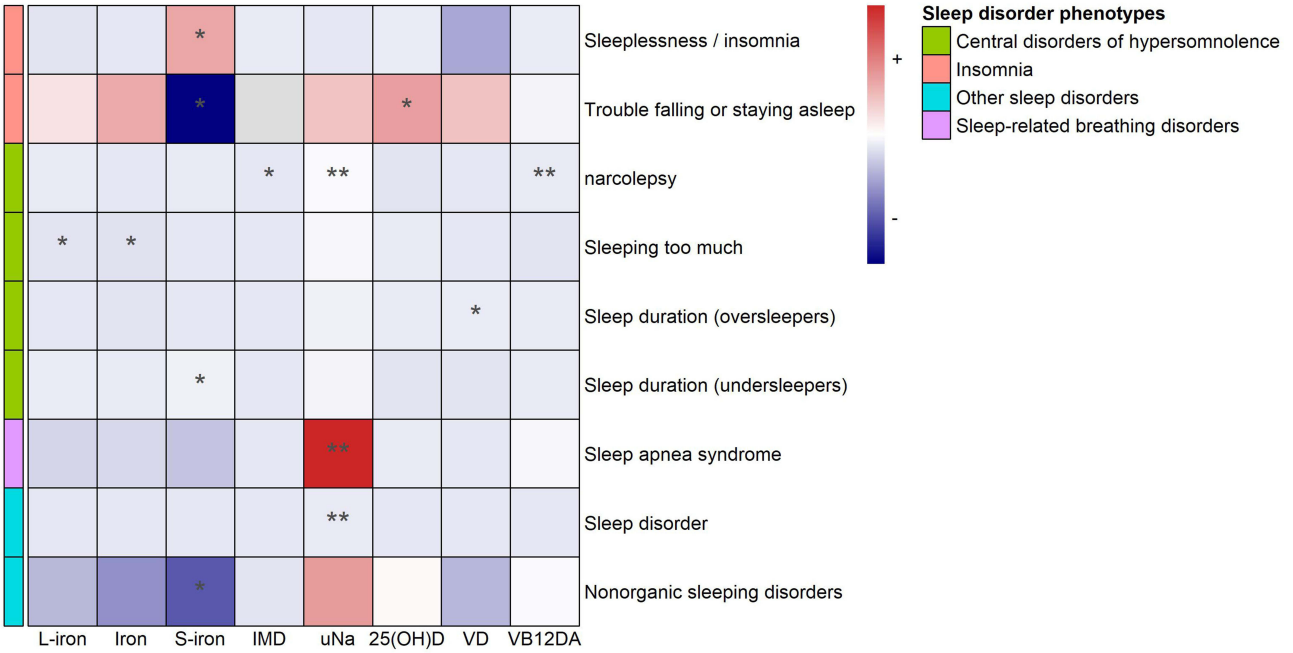

Figure 3 The genetically predicted micronutrient's effect on sleep disorder subtypes and the causal risk factors are gauged by the Z-scores of Mendelian randomization effect size estimates. The heatmap displayed color-coded lattices to signify the effect size (Z-score), with genetically predicted increased micronutrient level related to a higher risk of sleep disorder colored in red and lower risk of outcomes colored in blue. Darker shades on the heatmap correspond to greater effect sizes. *Indicates that the potential causal relationship was significant. *P < 0.05, **P < 0.01, ***P < 0.001. The abbreviations can be found in the Abbreviations.

Consistent with the aforementioned IVW outcomes, our MR analysis revealed significant genetic associations of 8 micronutrients with at least one type of sleep disorders (Figure 3). The absolute effect sizes, denoted as |β| (per 1 standard deviation increase in the genetically proxied exposure), ranged from 2.56×10^{-5} (lowest absolute value of the effect size) to 0.80 (highest absolute value of the effect sizes). Specifically, genetic estimations indicated that higher level of uNa was correlated with a heightened risk of narcolepsy, sleep apnea syndrome, sleep disorder, with the statistically significant associations marked by asterisks (Displayed in red in the image, Figure 3). In summary, the higher the IMD, uNa and VB12DA concentrations, the more likely narcolepsy was to occur. Moreover, the lower the L-iron and iron, the more likely sleep too much. Trouble falling or staying asleep was inversely related to S-iron concentration and positively related to serum 25(OH)D concentration. The relationship between other sleep disorder subtypes and single micronutrients was shown in Figure 3.

For the validity of MR-based causal inferences, it was crucial to verify that SNP-outcome associations are a direct consequence of the exposure of interest and not due to horizontal pleiotropy or other confounding pathways. In our investigation, we performed Cochrane's Q test, MR-Egger intercept, and leave-one-out sensitivity analyses to ascertain the pleiotropy resistance. (Supplementary Table 2, Supplementary Figure 2).

In addition to iron metabolism disorder (SNPs number less than 3), for the other seven micronutrients where there is an availability of three or more genetic instrumental variables, MR-Egger sensitivity analyses yielded consistent Mendelian randomization estimates to the main IVW analysis (Supplementary Table 1). This indicated that pleiotropic effects of genetic variants were probably not significantly biasing the Mendelian randomization estimates. Leave-one-out analysis also confirmed the stability of IVW causal estimations (Supplementary Figure 2). The analysis showed that in the univariable MR analyses, no specific SNP had a significant effect on altering the IVW causal estimation.

Discussion

Sleep was an essential neurophysiological condition and served as a foundation for both psychological and physiological wellness. Researchers recommended individuals should secure a minimum of 7 hours of sleep per night to optimize health and functionality.[28] Due to longer working hours, stressful lives, ambient light and prolonged screen usage, an

increasing proportion of individuals were experiencing limited sleep time. However, deviating from the recommended sleep time might seriously endanger health. Studies showed that in America, 26% of adults typically slept 6–7 hours a night, and 20% slept less than 6 hours.[29] 32.1% of Dutch adults have encountered problems related to the quality of their sleep, and 43.2% have expressed that they do not receive enough sleep.[30]

As mentioned above, sleep disorders were mainly divided into seven categories, which in turn contain many subtypes, such as insomnia were divided into chronic insomnia disorder, short-term insomnia disorder and other insomnia disorder; sleep-related breathing disorders were divided into obstructive sleep apnea disorders, central sleep apnea syndromes, sleep-related hypoventilation disorders, sleep-related hypoxemia disorder.[11] Additionally, many people might experience several subtypes of sleep disorders at once. Since the spring of 2020, the global COVID-19 pandemic had generally disrupted the sleep of the global population. A two-year study in Italy showed that COVID-19 led to a steady deterioration of sleep duration in the interviewees, particularly among younger people.[31]

More recently, micronutrient intake in sleep deprivation and sleep problems had gradually attracted attention. For instance, randomized controlled trials in infants found that infants who received zinc or iron supplements had longer night time and total sleep time compared to placebo group.[32] However, the causal relationship between micronutrient and sleep disorder was not yet fully understood.

As known, iron played an essential role in many important biological functions, including cognition and overall brain health. Iron homeostasis was critical for neurological health. Low iron levels were related to cognitive impairment, while high levels were related to Parkinson's disease (PD), Alzheimer's disease (AD), and other neurodegenerative diseases.[14] During sleep, people with obstructive sleep apnea had increased level of iron in the brain.[14] In our research, the relationship between iron levels at different sites and sleep disorders was distinguished. Both liver iron and serum iron were inversely related to sleeping too much. Nevertheless, spleen iron concentration was positively associated with sleeplessness/insomnia and sleep duration (undersleepers) and inversely related to trouble falling or staying asleep and nonorganic sleeping disorders (Figures 2 and 3). Therefore, we provided evidence for the potential causal relationship between iron content in different parts and different subtypes of sleep disorders using MR analysis.

At present, there were few studies on the impact of sodium ion on sleep, and the current research mainly focused on the effects of sodium leak channel and sodium oxybate in the treatment of sleep disorders. So far, the only available drug that might improve all major symptoms of narcolepsy was sodium oxybate.[33] According to the American Academy of Sleep Medicine, sodium oxybate, despite having unknown mechanisms of action, is recommended as first-line treatment for individuals suffering from excessive daytime sleepiness and cataplexy.[34] In our study, urine sodium levels were positively correlated with narcolepsy, sleep apnea syndrome and sleep disorders (Figures 2 and 3). Based on our current understanding, this should be the first time that Mendelian randomization had been used to demonstrate the relationship between urine sodium and various subtypes of sleep disorders.

Vitamin D (VD) was a fat-soluble vitamin that played an important role in several physiological processes, such as sleep patterns.[35] Research showed that VD deficiency was related to reduced sleep duration, poorer sleep quality, prolonged sleep onset times in pediatric populations.[35] But another study claimed an inverse connection between excessive daytime sleepiness and VD levels.[36] In other words, the more excessive daytime sleepiness, the lower the VD levels. However, compared to the healthy control group, patients with chronic insomnia exhibited notably decreased levels of VD.[37] Therefore, so far there was no clear and consistent conclusion on the connection between VD or 25(OH) D and sleep disorders. In our study, VD was directly proportional to sleep duration (oversleepers) and 25(OH)D was positively correlated to trouble falling or staying asleep (Figures 2 and 3), which implied new evidences for the causal relationship between VD or 25(OH)D and different subtypes of sleep disorders.

Despite vitamin B12 (VB12) acknowledged for its significance in the nervous system, research on the relationship between VB12 and sleep was still lacking.[38] Current research indicated manifold effects of VB12 on sleep patterns. A clinical trial reported a warning effect of VB12 supplementation on reduced sleep duration.[39] Another study revealed a distinct inverse correlation between serum VB12 concentrations and sleep duration among adults.[40] Additionally, young women with reduced consumption of VB12 tended to have later bedtimes.[41] RLS patients were significantly deficient in VB12 compared to healthy controls. But another study showed that decreased serum VB12 levels were independently related to the development of RLS, which demonstrated the

complex relationship between VB12 and RLS. Finally, a cross-sectional study showed that lower VB12 was linked to insomnia symptoms and sleepiness in select groups of individuals.[38] Therefore, The results demonstrated inconsistent effects of VB12 on sleep patterns. In our study, VB12DA was positively associated with narcolepsy (Figures 2 and 3), which support a causal effect between VB12DA and sleep disorder from the perspective of MR analysis.

In addition, it was imperative to clarify that our approach to identifying statistical significance was predicated on a P value of 5%, rather than post hoc tests that might be based on the number of outcome variables. Given the micronutrients and sleep disorder subtypes originated from separate databases, we have not applied multivariate MR; rather, we have undertaken a one-to-one analysis for each micronutrient and sleep disorder subtype, respectively. Figures 2 and 3 were just a combined presentation of the results from a one-to-one comparison of micronutrients and sleep disorder subtypes.

Since the MR study was based on three key assumptions, namely: SNPs were strongly correlated with exposure; SNPs were not correlated with known or unknown confounders; and SNPs affected outcomes only through exposure and not through other pathways.[18] However, this hypothesis cannot completely exclude the effect of confounding factors or horizontal pleiotropy, so this is one of the shortcomings of this study. In addition, our results were limited by the sample size. For example, we analyzed many other micronutrients (zinc, magnesium, calcium, iodine, selenium, copper, fluoride, lead and other vitamins) associations with sleep disorders, but no statistical differences emerged, which might be due to the small sample size. Meanwhile, our MR association effect size was relatively small, with a minimum value of 2.56×10^{-5}, and this might be caused by the small sample size and its clinical significance might be limited. Therefore, the conclusions of this research need to be validated in a larger sample size of the population. Moreover, the nine GWAS databases related to sleep disorders are from Europe, Africa and Asia (Table 2), which might lead to conclusions that cannot be generalized to the general population.

Conclusion

Our study should be the first to apply Mendelian randomization analysis at the SNP level to investigate the causal relationship between various micronutrients and different subtypes of sleep disorders, including L-iron and iron in sleeping too much, S-iron in sleeplessness/insomnia, trouble falling or staying asleep, sleep duration (undersleepers) and nonorganic sleeping disorders, IMD and VB12DA in narcolepsy, uNa in narcolepsy, sleep apnea syndrome and sleep disorder, VD in sleep duration (oversleepers), 25(OH)D in trouble falling or staying asleep. The study presents a novel insight into the impact of micronutrients on sleep disorders, providing a groundwork for future pharmaceutical advancements and clinical applications.

Abbreviations

GWAS, genome-wide association studies; 25(OH)D, 25-Hydroxyvitamin D; ICSD-3, International Classification of Sleep Disorders; IMD, iron metabolism disorder; IVW, inverse variance weighted; LD, linkage disequilibrium; L-iron, liver iron content; MR, Mendelian randomization; MR-Egger, Mendelian randomization Egger regression; OR, odds ratio; REM, rapid eye movement; RLS, restless legs syndrome; S-iron, spleen iron concentration; uNa, urine sodium; VB12DA, vitamin B12 deficiency anaemia; VD, vitamin D.

Data Sharing Statement

The data supporting the findings of this study are available on the IEU open GWAS.

Ethics Declaration

This research was conducted in compliance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Beijing Neurosurgical Institute.

Acknowledgments

We are grateful to the extensive group of participants and researchers for gathering the GWAS data and the accessibility of their GWAS summary statistics to the public.

Funding

This work was sponsored by the CCMU-Haier YKLIFE Innovative Elite Talent Program (No. 2024KCJY0412) and supported by grants from the National Natural Science Foundation of China (No. 81901196), Research and Cultivation Foundation of Beijing Neurosurgical Institute (No. 2023 Reform and Development-Institute Nature-3).

Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Xiao X, Rui YM, Jin Y, et al. Relationship of sleep disorder with neurodegenerative and psychiatric diseases: an updated review. *Neurochem Res*. 2024;49(3):568–582.PMID: 38108952. doi:10.1007/s11064-023-04086-5
2. Marafioti G, Corallo F, Cardile D, et al. REM sleep behavior disorder and cognitive functions in Parkinson's patients: a systematic review. *J Clin Med*. 2023;12(23):7397.PMID: 38068449. doi:10.3390/jcm12237397
3. Desana K, Thom SL, Aafje D, et al. Sleep characteristics across the lifespan in 1.1 million people from the Netherlands, United Kingdom and United States: a systematic review and meta-analysis. *Nat Hum Behav*. 2021;5(1):113–122.PMID: 33199855. doi:10.1038/s41562-020-00965-x
4. Endomba FT, Tchebegna PY, Chiabi E, et al. Epidemiology of insomnia disorder in older persons according to the diagnostic and statistical manual of mental disorders: a systematic review and meta-analysis. *Eur Geriatr Med*. 2023;14(6):1261–1272.PMID: 37725311. doi:10.1007/s41999-023-00862-2
5. O'Driscoll DM, Young AC. Contemporary concise review 2022: sleep. *Respirology*. 2023;28(6):518–524.PMID: 36990762. doi:10.1111/resp.14500
6. Vizzini L, Popovic M, Zugna D, et al. Maternal anxiety, depression and sleep disorders before and during pregnancy, and preschool ADHD symptoms in the NINFEA birth cohort study. *Epidemiol Psychiatr Sci*. 2019;28(5):521–531.PMID: 29665879. doi:10.1017/S2045796018000185
7. Maust DT, Solway E, Clark SJ, et al. Prescription and nonprescription sleep product use among older adults in the United States. *Am J Geriatr Psychiatry*. 2019;27(1):32–41.PMID: 30409547. doi:10.1016/j.jagp.2018.09.004
8. Chery MJ, Baral A, Rolle LD, et al. Depression, sleep health & sociodemographic correlates in a nationwide survey: implications for depression treatment during the covid-19. *Nat Sci Sleep*. 2024;16:17–31. PMID: 38235481. doi:10.2147/NSS.S434148
9. Liu WK, Kothare S, Jain S. Sleep and epilepsy. *Semin Pediatr Neurol*. 2023;48:101087. PMID: 38065633. doi:10.1016/j.spen.2023.101087
10. Li YJ, Que MX, Wang X, et al. Exploring astrocyte-mediated mechanisms in sleep disorders and comorbidity. *Biomedicines*. 2023;11(9):2476. PMID: 37760916. doi:10.3390/biomedicines11092476
11. Sateia MJ. International classification of sleep disorders-third edition: highlights and modifications. *Chest*. 2014;146(5):1387–1394.PMID: 25367475. doi:10.1378/chest.14-0970
12. Jiang H. Hypothalamic GABAergic neurocircuitry in the regulation of energy homeostasis and sleep/wake control. *Med Rev*. 2022;2(5):531–540. PMID: 37724165. doi:10.1515/mr-2022-0022
13. Peuhkuri K, Sihvola N, Korpela R. Diet promotes sleep duration and quality. *Nutr Res*. 2012;32(5):309–319.PMID: 22652369. doi:10.1016/j.nutres.2012.03.009
14. Marchi NA, Pizzarotti B, Solelhac G, et al. Abnormal brain iron accumulation in obstructive sleep apnea: a quantitative MRI study in the HypnoLaus cohort. *J Sleep Res*. 2022;31(6):e13698.PMID: 35830960. doi:10.1111/jsr.13698
15. Deng MG, Liu F, Liang YH, et al. Associations of serum zinc, copper, and selenium with sleep disorders in the American adults: data from NHANES 2011-2016. *J Affect Disord*. 2023;323:378–385. PMID: 36464094. doi:10.1016/j.jad.2022.11.088
16. Laleli KB, Elmas B, Tugrul ED, et al. Evaluation of serum selenium level, quality of sleep, and life in pregnant women with restless legs syndrome. *Biol Trace Elem Res*. 2023;201(3):1143–1150.PMID: 36255554. doi:10.1007/s12011-022-03447-5
17. Tutan D, Ulfberg J, Aydemir N, et al. The Relationship between serum selenium levels and restless leg syndrome in chronic kidney disease patients. *Medicina*. 2023;59(10):1795.PMID: 37893513. doi:10.3390/medicina59101795
18. Zhang HP. Pros and cons of Mendelian randomization. *Fertil Steril*. 2023;119(6):913–916.PMID: 36990264. doi:10.1016/j.fertnstert.2023.03.029
19. Boehm FJ, Zhou X. Statistical methods for Mendelian randomization in genome-wide association studies: a review. *Comput Struct Biotechnol J*. 2022;20:2338–2351. PMID: 35615025. doi:10.1016/j.csbj.2022.05.015
20. Larsson SC, Mason AM, Vithayathil M, et al. Circulating vitamin C and digestive system cancers: Mendelian randomization study. *Clin Nutr*. 2022;41(9):2031–2035.PMID: 35986965. doi:10.1016/j.clnu.2022.07.040
21. Liu Y, Basty N, Whitcher B, et al. Genetic architecture of 11 organ traits derived from abdominal MRI using deep learning. *Elife*. 2021;10:e65554. PMID: 34128465. doi:10.7554/eLife.65554
22. Benyamin B, Esko T, Ried JS, et al. Novel loci affecting iron homeostasis and their effects in individuals at risk for hemochromatosis. *Nat Commun*. 2014;5:4926. PMID: 25352340. doi:10.1038/ncomms5926
23. Sorokin EP, Basty N, Whitcher B, et al. Analysis of MRI-derived spleen iron in the UK Biobank identifies genetic variation linked to iron homeostasis and hemolysis. *Am J Hum Genet*. 2022;109(6):1092–1104.PMID: 35568031. doi:10.1016/j.ajhg.2022.04.013
24. Mbatchou J, Barnard L, Backman J, et al. Computationally efficient whole-genome regression for quantitative and binary traits. *Nat Genet*. 2021;53(7):1097–1103.PMID: 34017140. doi:10.1038/s41588-021-00870-7

25. Barton AR, Sherman MA, Mukamel RE, et al. Whole-exome imputation within UK Biobank powers rare coding variant association and fine-mapping analyses. *Nat Genet*. 53(8):1260–1269. PMID: 34226706. doi:10.1038/s41588-021-00892-1
26. Sakaue S, Kanai M, Tanigawa Y, et al. A cross-population atlas of genetic associations for 220 human phenotypes. *Nat Genet*. 2021;53 (10):1415–1424.PMID: 34594039. doi:10.1038/s41588-021-00931-x
27. Jones SE, Tyrrell J, Wood AR, et al. Genome-wide association analyses in 128,266 individuals identifies new morningness and sleep duration loci. *PLoS Genet*. 2016;12(8):e1006125.PMID: 27494321. doi:10.1371/journal.pgen.1006125
28. Grandner MA, Alfonso-Miller P, Fernandez-Mendoza J, et al. Sleep: important considerations for the prevention of cardiovascular disease. *Curr Opin Cardiol*. 2016;31(5):551–565.PMID: 27467177. doi:10.1097/HCO.0000000000000324
29. Hirshkowitz M, Whiton K, Albert SM, et al. National Sleep Foundation's sleep time duration recommendations: methodology and results summary. *Sleep Health*. 2015;1(1):40–43.PMID: 29073412. doi:10.1016/j.sleh.2014.12.010
30. Monteiro LZ, de Farias JM, de Lima TR, et al. Physical activity and sleep in adults and older adults in Southern Brazil. *Int J Environ Res Public Health*. 2023;20(2):1461.PMID: 36674217. doi:10.3390/ijerph20021461
31. Salfi F, Amicucci G, Corigliano D, et al. Two years after lockdown: longitudinal trajectories of sleep disturbances and mental health over the COVID-19 pandemic, and the effects of age, gender and chronotype. *J Sleep Res*. 2023;32(3):e13767.PMID: 36317491. doi:10.1111/jsr.13767
32. Kordas K, Siegel EH, Olney DK, et al. The effects of iron and/or zinc supplementation on maternal reports of sleep in infants from Nepal and Zanzibar. *J Dev Behav Pediatr*. 2009;30(2):131–139.PMID: 19322104. doi:10.1097/DBP.0b013e31819e6a48
33. Lammers GJ. Drugs used in narcolepsy and other hypersomnias. *Sleep Med Clin*. 2018;13(2):183–189.PMID: 29759269. doi:10.1016/j.jsmc.2018.02.009
34. Ono T, Takenoshita S, Nishino S. Pharmacologic management of excessive daytime sleepiness. *Sleep Med Clin*. 2022;17(3):485–503.PMID: 36150809. doi:10.1016/j.jsmc.2022.06.012
35. Mao R, Zhou GW, Jing DR, et al. Vitamin D status, Vitamin D receptor polymorphisms, and the risk of incident rosacea: insights from Mendelian randomization and cohort study in the UK Biobank. *Nutrients*. 2023;15(17):3803.PMID: 37686836. doi:10.3390/nu15173803
36. McCarty DE, Reddy A, Keigley Q, et al. Vitamin D, race, and excessive daytime sleepiness. *J Clin Sleep Med*. 2012;8(6):693–697.PMID: 23243403. doi:10.5664/jcsm.2266
37. Zhao K, Luan XQ, Liu YT, et al. Low serum 25-hydroxyvitamin D concentrations in chronic insomnia patients and the association with poor treatment outcome at 2 months. *Clin Chim Acta*. 2017;475:147–151. PMID: 29080688. doi:10.1016/j.cca.2017.10.024
38. Bouloukaki I, Lampou M, Raouzaiou KM, et al. Association of Vitamin B12 levels with sleep quality, insomnia, and sleepiness in adult primary healthcare users in Greece. *Healthcare*. 2023;11(23):3026.PMID: 38063594. doi:10.3390/healthcare11233026
39. Mayer G, Kroger M, Meier-Ewert K. Effects of vitamin B12 on performance and circadian rhythm in normal subjects. *Neuropsychopharmacology*. 1996;15(5):456–464.PMID: 8914118. doi:10.1016/S0893-133X(96)00055-3
40. Beydoun MA, Gamaldo AA, Canas JA, et al. Serum nutritional biomarkers and their associations with sleep among US adults in recent national surveys. *PLoS One*. 2014;9(8):e103490.PMID: 25137304. doi:10.1371/journal.pone.0103490
41. Ji XP, Grandner MA, Liu JH. The relationship between micronutrient status and sleep patterns: a systematic review. *Public Health Nutr*. 2017;20 (4):687–701.PMID: 27702409. doi:10.1017/S1368980016002603