Nature and Science of Sleep

Elevate Series

Open Access Full Text Article

ORIGINAL RESEARCH

Getting Up in the Morning and Chronotype in Relation to Polycystic Ovarian Syndrome: A Mendelian Randomization and Cross-Sectional Study

Diliqingna Dilimulati[1,*], Jiayi Lu[2,*], Jinghua Li[2,*], Meili Cai[1], Yuqin Zhang[1], Xiaowen Shao[3], Haibing Chen[1], Qian Wan[4], Fang He[4], Chaoyan Yue[5], Manna Zhang[1,6], Shen Qu[1]

[1]Department of Endocrinology and Metabolism, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, 200072, People's Republic of China; [2]Department of Clinical Laboratory, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, 200072, People's Republic of China; [3]Department of Obstetrics and Gynecology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, 200072, People's Republic of China; [4]Department of Endocrinology and Metabolism, The First People's Hospital of Chenzhou, Chenzhou, Hunan, 423000, People's Republic of China; [5]Department of Laboratory Medicine, Obstetrics and Gynecology Hospital of Fudan University, Shanghai, 200011, People's Republic of China; [6]Department of General Practice, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, 200072, People's Republic of China

*These authors contributed equally to this work

Correspondence: Manna Zhang, Department of Endocrinology and Metabolism, Shanghai Tenth People's Hospital, No. 301, Middle Yanchang Road, Jingan District, Shanghai, 200072, People's Republic of China, Tel +86 13774448495, Email mannazhang@126.com; Chaoyan Yue, Department of Laboratory Medicine, Obstetrics and Gynecology Hospital of Fudan University, No. 419, Fangxie Road, Huangpu District, Shanghai, 200011, People's Republic of China, Tel +86 02133189900, Email 20111250007@fudan.edu.cn

Background: Although a connection between circadian rhythm and polycystic ovarian syndrome (PCOS) has been shown in previous studies, the exact cause of this association is not well understood.

Purpose: This study aimed to use Mendelian randomization (MR) method to analyze the potential association between getting up in the morning and chronotype with PCOS, and a cross-sectional study was conducted to further validate these results.

Methods: Using summary information from large-scale genome-wide association studies (GWASs) in people of European ancestry, we conducted univariable MR (UVMR) and multivariable MR (MVMR) analyses to examine the causal effect of genetically determined getting up in the morning and chronotype on PCOS. We also investigated the association between wake-up time and sleep midpoint with the risk of PCOS and total testosterone (TT) levels in a cohort of 777 women of reproductive age.

Results: Our findings indicate a causal relationship between the genetic prediction of getting up in the morning and chronotype with a reduced incidence of PCOS. In a cross-sectional study, a sleep midpoint of > 4:00 was linked to a higher risk of PCOS and increased TT levels than a sleep midpoint of < 3:30. In women with a BMI < 24 kg/m^2, earlier wake-up times and sleep midpoints were associated with a lower risk of PCOS and decreased TT levels.

Conclusion: This study indicates that a genetic predisposition to getting up in the morning and chronotype are linked to a reduced risk of PCOS. Additionally, earlier wake-up times and sleep midpoints are associated with a lower risk of PCOS and decreased TT levels.

Keywords: polycystic ovarian syndrome, getting up in the morning, chronotype, Mendelian randomization

Introduction

Polycystic ovarian syndrome (PCOS), the prevailing endocrine disorder, affects approximately 10–13% of women of reproductive age worldwide.[1] According to Rotterdam criteria, PCOS is diagnosed based on the presence of at least two of the following: irregular menstrual cycles (oligo-ovulation or anovulation), hyperandrogenism, and polycystic ovarian morphology.[2] Women with PCOS have an increased risk of insulin resistance (IR), obesity, infertility, and type 2 diabetes mellitus (T2DM).[3,4]

Received: 18 December 2024
Accepted: 11 April 2025
Published: 1 May 2025

The exact etiology of PCOS is unknown and may involve behavioral patterns, intrauterine environments, and genetics.[5] In population studies, individuals with PCOS experienced more frequent sleep disruptions such as circadian rhythm disturbances, short sleep duration, snoring, and sleepiness.[6–8] Morning circadian misalignment has been linked to metabolic dysregulation.[9] An observational study found that women with a lower chronotype score had an increased risk of developing PCOS and exhibited increased testosterone levels, body mass index (BMI), and homeostasis model of insulin resistance (HOMA-IR).[10] Previous research has indicated that chronotype, represented by the sleep midpoint, correlates with higher postprandial glucose levels,[11] and an increased risk of gestational diabetes in pregnant women.[12] However, this relationship has not yet been explored in PCOS. From a physiological perspective, the follicular growth processes in mammalian ovaries exhibit strong circadian rhythms that are synchronized with endocrine signals and light cycles.[13] Ovarian-specific rhythms depend on the precise regulation of the rhythmic expression of clock-controlled genes. Examples of clock-controlled genes include the steroidogenic acute regulatory protein (STAR) and the peroxisome proliferator–activated receptor gamma coactivator 1-alpha (PPARGC1A). Additionally, ovarian-specific rhythms depend on the regulation of core clock genes, such as CLOCK and BMAL1.[14–16] In summary, the aforementioned studies did not establish a causal link between getting up in the morning, chronotype (circadian tendency for morningness in biological and behavioral rhythms), and PCOS, highlighting the current research gap in this area.

Mendelian randomization (MR) analysis can be used to fill these research gaps. By using genetic variations as instrumental variables (IVs) to ascertain the causal links between exposures and outcomes, MR provides a fresh viewpoint for epidemiological research.[17,18] MR overcomes the shortcomings of observational studies in demonstrating causality and reducing the impact of potential confounding factors on result accuracy because of the random distribution of alleles at birth, which makes MR equivalent to the principles of randomized controlled trials.[19] Previous studies have explored the connection between sleep and chronic conditions, such as T2DM and cardiovascular disease, using MR Methods,[20,21] but research on sleep traits and PCOS is still lacking.

This study is composed of two main components: 1) MR analysis to investigate whether getting up in the morning and chronotype had a causal relationship with PCOS, and 2) an observational study to validate the MR results by examining the association between various wake-up times and sleep midpoints, and the occurrence of PCOS and total testosterone (TT) levels in participants with and without PCOS. This is crucial for understanding the causal relationship between getting up in the morning, chronotype, and PCOS, and helps to identify prevention and treatment strategies for PCOS.

Materials and Methods

Study Design

Figure 1 shows an overview of the study design and the assumptions of the MR design. Our study consisted of two parts: an MR analysis and observational studies. First, we analyzed the relationship between genetically determined getting up in the morning and PCOS. We then analyzed the relationship between the chronotype and PCOS. The main results were obtained using the inverse variance weighted (IVW) random-effects model and multivariable MR (MVMR). To validate the MR analysis results, we recruited women with PCOS and controls to examine the association between wake-up time, sleep midpoint, PCOS risk, and TT level.

Data Sources

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology Using MR (STROBE-MR) guidelines (Supplementary Table S1). Online public data sources for getting up in the morning, chronotypes, and PCOS were obtained (Supplementary Table S2). Previously published SNPs for each phenotype were selected as IVs. All participants in our data pool for MR analysis were of European ethnicity. Public data related to MR have obtained relevant informed consent and ethical approval; therefore, no additional ethical approval was required for this part of the study. This study meets the exemption from full ethics review based on item 1 and 2 of Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects dated February 18, 2023, China. This study complies with the principles of the Declaration of Helsinki, and the Ethics Committee of Shanghai Tenth People's Hospital approved the cohort study protocol (SHSY-IEC 4.1/21-227/01), which was registered with ClinicalTrials.gov (NCT05063383).

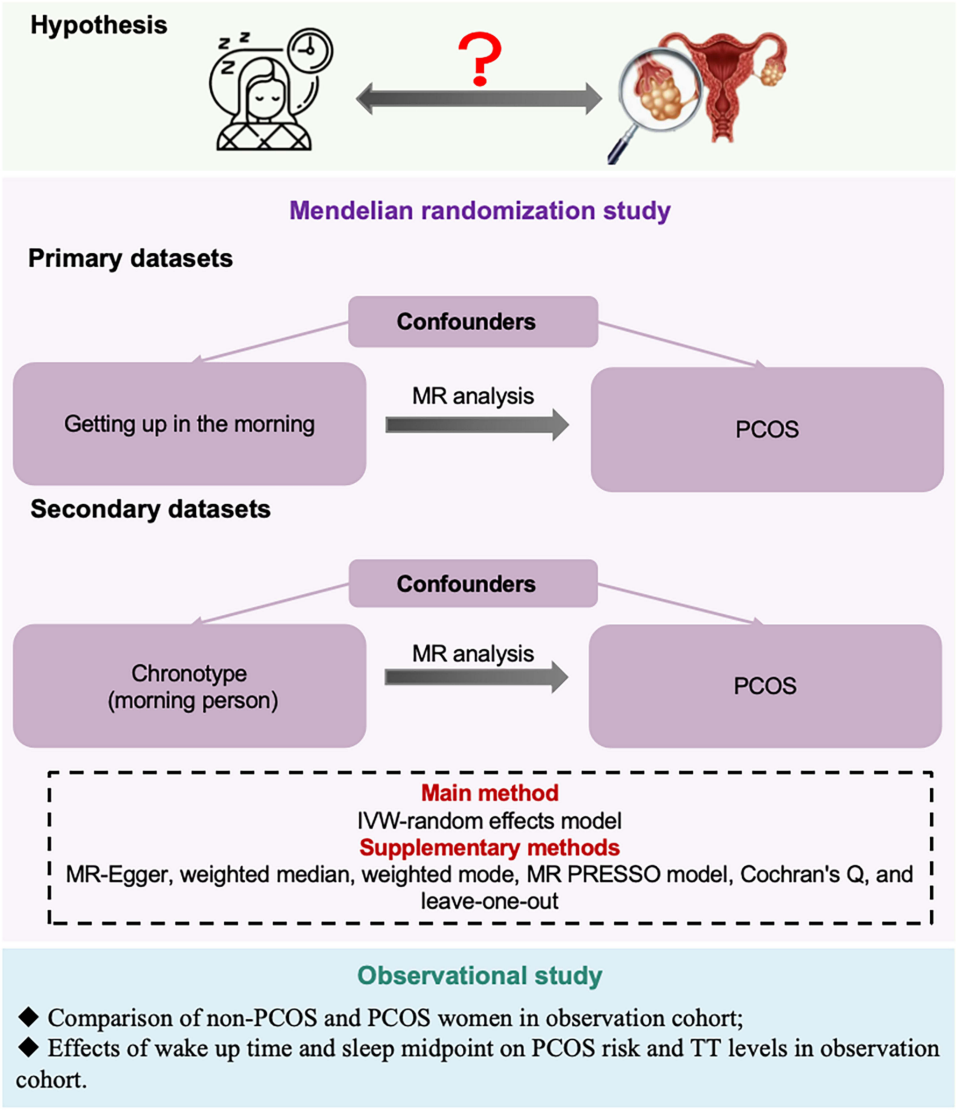

Figure 1 Study design overview and assumptions of the MR design.
Notes: MR requires the use of reliable genetic instrumental variables that meet three essential assumptions. They are Assumption 1 (generic variants should predict exposure); Assumption 2 (generic variants must remain independent of confounding factors); and Assumption 3 (generic variants only affect the outcome through exposure, not through other pathways).
Abbreviations: IVW, inverse-variance weighted; MR, Mendelian randomization; PCOS, polycystic ovary syndrome; TT, total testosterone.

Genome-wide association studies (GWASs) summary statistics for getting up in the morning were obtained from an Open GWAS database published in 2017 by Neale Labs, which included GWAS data from 336,501 participants of European descent. GWAS summary statistics for chronotypes were obtained from the UK Biobank study (ID: ebi-a-GCST003837), encompassing 127,898 participants (https://gwas.mrcieu.ac.uk/).[22] These GWAS studies have used samples from the UK Biobank, a large population-based prospective cohort. The GWAS summary statistics for PCOS, identified as "finngen_R10_E4_PCOS", were sourced from the Finnish biobanks (https://finngen.gitbook.io/documentation/) and encompassed data from 226,869 individuals. The 10-year FinnGen study aims to gather and examine health and genetic information from half a million members of the Finnish Biobank. The most recent R10 dataset version was made available by the FinnGen team on December 18, 2023. GWAS summary statistics for BMI were sourced from the UK Biobank study, which included 454,884 participants (ID: ukb-b-2303).

Instrumental Variable Selection

In this study, genetic variation qualifies as an instrumental variable if it meets the following criteria: (1) SNPs must exhibit a strong association with exposure, (2) SNPs should not be associated with any confounding factors between exposure and outcome, and (3) SNPs must influence the outcome solely through exposure.

First, we identified SNPs from previously published data that showed a significant association with sleep traits (getting up in the morning and chronotype) and PCOS. We used $P < 5\times10^{-8}$ as the primary screening criterion for analyzing sleep traits as the exposure factor, and PCOS as the outcome. We removed SNPs in linkage disequilibrium ($r^2 < 0.001$, clumping window = 10,000 kb) to ensure independence of the exposure instruments. SNPs that were absent were eliminated from the database. Ultimately, IVs were identified as valid SNPs that were highly correlated with exposure. Additionally, F-value calculations were conducted to address potential bias from weak IVs, and SNPs with insufficient power ($F < 10$) were excluded. To calculate the F statistic, we used the formula $F = R^2 (n-k-1)/[k (1-R^2)]$. The R^2 value indicates the extent to which the instrumental variable explains the exposure. Next, we reconciled the exposure and outcome data, indicating that the same allele was responsible for both the exposure and resulting effects of the SNP. Finally, to maintain the uniformity of their effect alleles, we eliminated non-uniformly oriented echo SNPs using a harmonization procedure.

Study Participants of Observational Study

This case-control study included 670 PCOS patients and 107 healthy controls admitted to the Department of Endocrinology and Metabolism at the Shanghai Tenth People's Hospital between September 2019 and June 2024. Figure 2 shows the flow diagram. All participants signed informed consent forms following approval of the study procedure by the Shanghai Tenth People's Hospital Ethics Committee. According to Chinese guideline,[23] BMI was used

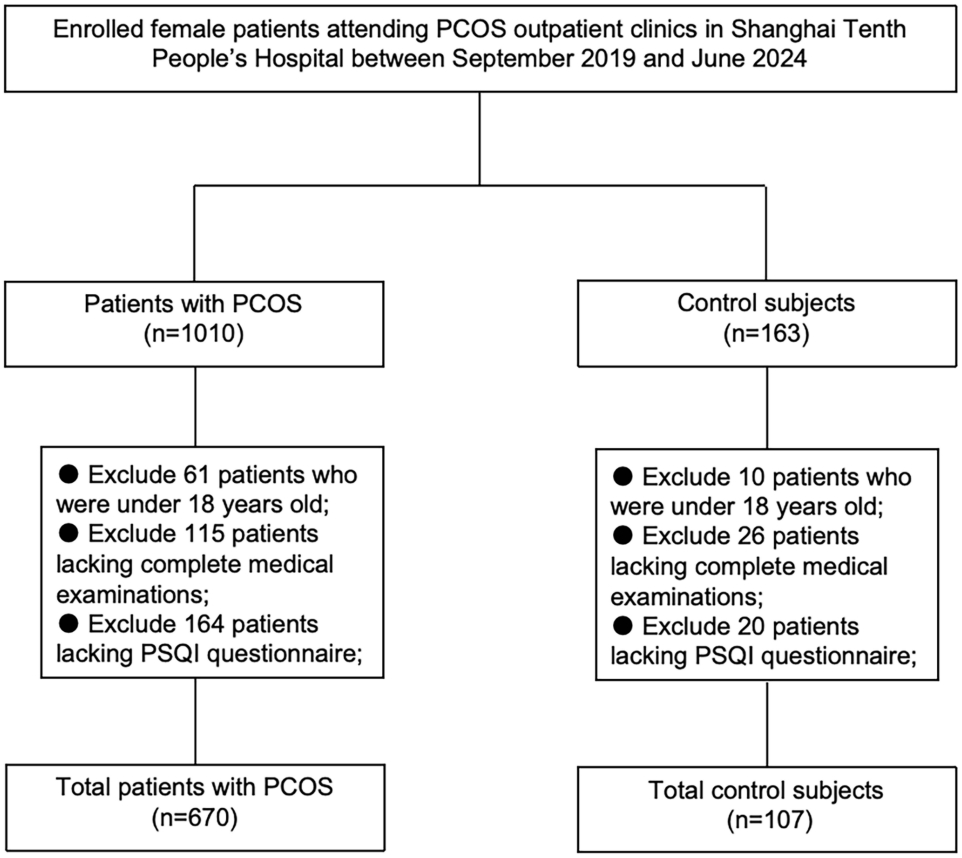

Figure 2 The flow diagram of patient recruitment and grouping.
Abbreviations: PCOS, polycystic ovary syndrome; PSQI, Pittsburgh Sleep Quality Index.

to categorize individuals into normal weight (BMI < 24 kg/m^2) and overweight or obese (BMI ≥ 24 kg/m^2) groups. The 2003 Rotterdam criteria were used to diagnose PCOS, requiring at least two of the following: 1) oligo- or anovulation, 2) biochemical or clinical signs of hyperandrogenism (eg, hirsutism and acne), and 3) polycystic ovaries, defined as ovarian volume >10 mL and/or ≥12 follicles per ovary, each 2–9 mm in diameter.[24] Furthermore, none of the PCOS patients who met the aforementioned requirements for inclusion criteria received treatment. A total of 670 women aged 18–45 years with PCOS were admitted to the Department of Endocrinology and Metabolism. The control group included 107 women aged 18–45 years with regular menstruation (21–35 days, lasting 2–8 days, with 20–60 mL of flow), no biochemical or clinical signs of hyperandrogenism, and no polycystic ovary symptoms on gynecologic ultrasonography. Exclusion criteria for PCOS patients and control subjects included (1) age under 18 or over 45 years, (2) severe liver or kidney dysfunction, (3) incomplete sleep questionnaire, (4) mental illness, and (5) secondary obesity due to other endocrine diseases.

Anthropometric Evaluation and Laboratory Examination

The age, weight, height, and menstrual status of each patient were measured and documented by a qualified endocrinologist. Weight/(height × height) (kg/m^2) is the formula used to calculate the BMI. After a minimum of ten hours of fasting, blood samples were collected in the morning. The TT levels were measured using an electrochemical luminescence immunoassay (Roche Diagnostics GmbH, Cot., Germany).

Wake-up Time and Sleep Midpoint

The Pittsburgh Sleep Quality Index (PSQI) was developed in 1989 by Dr. Buysse et al, psychiatrists at the University of Pittsburgh, to assess an individual's sleep quality.[25] PSQI questionnaire consists of a set of 19 items/questions organized to produce scores for 7 components. It is widely regarded as a reliable tool for evaluating sleep, and the validity of the self-reported PSQI questionnaire has been supported by prior research.[25] The questions "During the past month, when have you usually gone to bed at night?" and "In the past month, what time have you typically woken up in the morning?" were used to evaluate sleep information. Individuals were categorized into three groups based on their wake-up time: morning (before 7:00 AM), intermediate (7:00 AM-9:00 AM), and evening (after 9:00 AM). The chronotype was evaluated using the sleep midpoint, which considers the timing and length of sleep, with a later sleep midpoint suggesting a greater likelihood of being an evening person.[26] In the data analysis, the midpoint of the sleep phase, recorded in hours and minutes, was treated as a continuous variable on an hourly scale (eg, 3:30 = 3.5 h). Individuals were categorized into three groups based on their sleep midpoint: morning (before 3:30 AM), intermediate (3:30 AM - 4:00 AM), and evening (after 4:00 AM).

Statistical Analysis

Based on the previous study, the proportion of wake-up time >7:00 in the non-PCOS group was 55.7%.[27] According to a web-based calculator (MedSci Sample Size tools), with the assumption of the proportion of wake-up time >7:00 in the PCOS group = 70.7% alpha = 0.05, beta = 0.80, case-control ratio=6:1, the sample size required for the PCOS and non-PCOS group were 519 and 87, respectively.

Inverse-variance weighted (IVW), MR-Egger, weighted median, and weighted mode methods were used for the MR estimates. The IVW method is the preferred primary MR approach for summarizing data involving multiple uncorrelated genetic variants. The IVW method requires all IVs to be valid and unbiased if directional pleiotropy is absent. Other complementary MR methods (MR-Egger, weighted median, weighted mode, and MR pleiotropy) were used to control horizontal pleiotropy. MR-Egger regression can detect directional pleiotropy, but it has less statistical power.[28] Unlike IVW analysis, this method sets a nonzero intercept, permitting an unbalanced or directional average net horizontal pleiotropic effect across all SNPs. The weighted median method provides a robust estimate of causality if more than 50% of the weighted value is derived from effective IVs. MR-PRESSO can correct horizontal pleiotropy via outlier removal (outlier SNPs). Moreover, we conducted Cochran's Q statistic calculation, MR-Egger intercept test, and leave-one-out analysis to evaluate the presence of heterogeneity and pleiotropy.[29,30] MVMR represents the development of MR that considers feature variety. In this study, we employed MVMR to evaluate the potential associations between wake-up time, chronotype, and PCOS when adjusting for BMI.

For all normally distributed continuous variables, the results are expressed as mean and standard deviation, and median and interquartile range for data that were abnormally distributed continuous variables. Percentages were used to represent categorical variables. An independent samples *t*-test was performed to compare continuous variables between the two groups. Categorical variables were analyzed using Fisher's exact test or the chi-square test when applicable. The associations between wake-up time, sleep midpoint, and PCOS were examined using multiple logistic regression, with or without adjustment for age and BMI. With or without adjustment for age and BMI, multivariate linear regression was used to analyze the relationships among wake-up time, sleep midpoint, and TT level. Regression coefficients and 95% confidence intervals (CI) were calculated. BMI was used as a stratification factor, and sensitivity analysis was performed between subgroups to determine whether there was a consistent association between wake-up time and sleep midpoint, TT levels, and PCOS risk across subgroups. Sensitivity analyses were adjusted for age and BMI in BMI subgroups. Interaction tests were performed to assess the ORs or β values between the analyzed subgroups.

Statistical analyses were performed by using SPSS 25.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as $P < 0.05$. Analyses were conducted using R (v4.3.0), two-sample MR (v0.4.25), and MR PRESSO (v1.0) packages. The "Mendelian Randomization" R package was utilized to carry out our MVMR.

Results

Two-Sample MR Analysis

Initially, we conducted a causality assessment using getting up in the morning as an exposure factor, and PCOS as an outcome factor (Figure 3). The summary information for instrumental variables were listed in Supplementary Tables S3 and S4. Using univariable MR (UVMR) analysis, our findings indicated that getting up in the morning (OR = 0.316, 95% CI = 0.102 to 0.978, P = 0.046) was causally associated with a decreased incidence of PCOS. Getting up in the morning was found to be causally associated with a decrease in the incidence of PCOS (OR = 0.209, 95% CI = 0.071 to 0.617, P = 0.005) in the MVMR.

Next, we conducted a causal analysis with PCOS as outcome factors, and chronotype as an exposure factor (Figure 4). Genetic prediction of chronotype was causally associated with a decreased incidence of PCOS (UVMR: β = 0.277, 95% CI = 0.090 to 0.850, P = 0.025).

The primary IVW estimate was consistent with the MR-Egger, weighted median, and weighted mode results (Supplementary Tables S5 and S6). Supplementary Figures S1 and S2 display the forest plot, leave-one-out analysis, scatter plot, and funnel plot for all the analyses. Leave-one-out analysis indicated that no individual SNP affected overall estimates. The MR-Egger regression intercept showed no pleiotropy and the symmetry of the funnel plots validated our MR analysis. Although Cochran's Q test ($P < 0.05$) indicated some heterogeneity in the results, it was acceptable, as the IVW approach was the primary outcome of this study and did not invalidate the MR estimates.

Cohort Study

Table 1 shows 670 PCOS patients and 107 control individuals from the observational study. Compared with the control group, PCOS patients were younger ($P < 0.001$), had a higher BMI ($P = 0.001$), higher total testosterone levels ($P < 0.001$), and had fewer annual menstrual cycles ($P < 0.001$). In the control group, 33.64% (36/107) of women's wake-

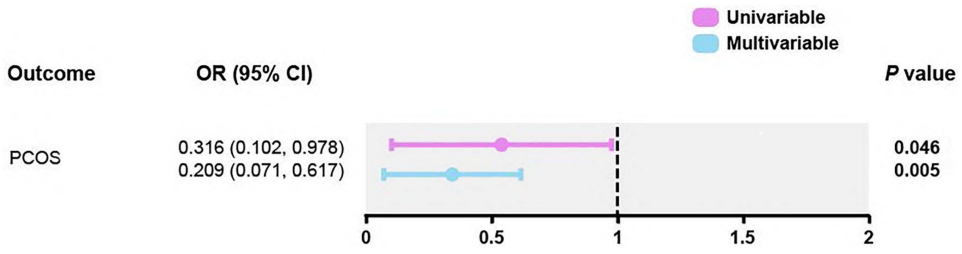

Figure 3 MR of getting up in the morning and risk of polycystic ovary syndrome (PCOS). The bold font indicated P < 0.05.
Abbreviation: PCOS, polycystic ovary syndrome.

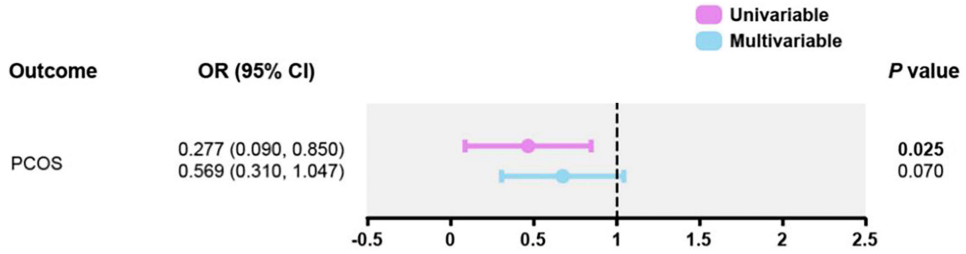

Figure 4 MR of chronotype and risk of polycystic ovary syndrome (PCOS). The bold font indicated P < 0.05.
Abbreviation: PCOS, polycystic ovary syndrome.

up times were < 7:00, 57.01% (61/107) were 7:00–9:00, and 9.35% (10/107) were > 9:00. Among women with PCOS, 20.00% (134/670) of women's wake-up times were < 7:00, 65.97% (442/670) of women's wake-up time were 7:00–9:00, 14.03% (94/670) of women's wake-up time was > 9:00.

The results of multiple logistic regression analysis of the effects of wake-up time and sleep midpoint on PCOS risk and TT levels are shown in Table 2. Compared with the wake-up time < 7:00, the risk of PCOS was increased in women with a wake-up time of 7:00–9:00 (OR = 1.95, 95% CI 1.24 to 3.07, P = 0.004) and > 9:00 (OR = 2.53, 95% CI 1.19 to 5.34, P = 0.015). This difference was not significant after adjusting for age and BMI. Compared with women with a sleep midpoint < 3:30, those with a sleep midpoint > 4:00 (OR = 2.75, 95% CI 1.68 to 4.48, P < 0.001) had an increased risk of PCOS. The difference remained significant after adjusting for age and BMI (OR = 1.91, 95% CI 1.14 to 3.19, P = 0.014). Compared with women whose wake-up times were < 7:00, the wake-up time of 7:00–9:00 (β = 0.16, 95% CI 0.01 to 0.30, P = 0.032) and > 9:00 (β = 0.25, 95% CI 0.05 to 0.45, P = 0.015) were positively correlated with TT levels. However, this association was not significant after adjusting for age and BMI. Women with sleep midpoint < 3:30, women a sleep midpoint > 4:00 were associated with TT levels (β = 0.25, 95% CI 0.12 to 0.38, P = 0.001). After adjusting for age and BMI, there was still a positive correlation with TT levels (β = 0.16, 95% CI 0.03 to 0.30, P = 0.017).

After BMI grouping (Supplementary Tables S7 and S8), among women with a BMI < 24 kg/cm^2, the risk of PCOS increased in women with a wake-up time of 7:00–9:00 (OR = 2.95, 95% CI 1.65 to 5.25, P < 0.001) and > 9:00 (OR = 3.75, 95% CI 1.34 to 10.50, P = 0.012) compared to women with a wake-up time of < 7:00. However, after adjusting for age and BMI, the incidence of PCOS continued to increase in the 7:00–9:00 group (OR = 2.17, 95% CI 1.18 to 4.01, P = 0.013), and there was no significant OR value in the >9:00 group (OR = 2.38, 95% CI 0.82 to 6.96, P = 0.112). In the BMI < 24 kg/cm^2 group, women with a sleep midpoint > 4:00 had an increased risk of PCOS (OR = 3.22, 95% CI 1.68 to 6.16, P < 0.001)

Table 1 Baseline Characteristics of PCOS and Control Individuals

Items	Non-PCOS (n=107)	PCOS (n=670)	P values
Age	30.49 ± 5.54	27.34 ± 4.81	<0.001***
BMI (kg/m^2)	23.48 ± 4.67	25.18 ± 5.85	0.001**
TT (nmol/L)	1.09 ± 0.56	1.67 ± 0.76	<0.001***
Menstrual Cycles (no./yr)	11.88 ± 0.74	9.66 ± 3.38	<0.001***
Wake up time			0.005**
< 7:00	33.64% (36/107)	20.00% (134/670)	
7:00–9:00	57.01% (61/107)	65.97% (442/670)	
> 9:00	9.35% (10/107)	14.03% (94/670)	
Sleep midpoint			<0.001***
<3:30	54.21% (58/107)	33.28% (223/670)	
3:30–4:00	20.56% (22/107)	24.18% (162/670)	
>4:00	25.23% (27/107)	42.54% (285/670)	

Notes: **Indicated P<0.01; ***Indicated P<0.001.
Abbreviations: PCOS, polycystic ovary syndrome; BMI, body mass index; TT, total testosterone.

https://doi.org/10.2147/NSS.S511467

Table 2 Effects of Wake Up Time and Sleep Midpoint on PCOS Risk and TT Levels

Outcomes	Exposures	Non-Adjusted OR / β (95% CI)	*P*	Adjusted OR / β (95% CI)	*P*
PCOS	Wake up time				
	<7:00	Reference	/	Reference	/
	7:00–9:00	1.95 (1.24, 3.07)	0.004**	1.57 (0.96, 2.57)	0.071
	>9:00	2.53 (1.19, 5.34)	0.015*	1.49 (0.67, 3.27)	0.325
	Sleep midpoint				
	<3:30	Reference	/	Reference	/
	3:30–4:00	1.92 (1.13, 3.26)	0.164	1.67 (0.94, 2.97)	0.082
	>4:00	2.75 (1.68, 4.48)	<0.001***	1.91 (1.14, 3.19)	0.014*
TT levels	Wake up time				
	<7:00	Reference	/	Reference	/
	7:00–9:00	0.16 (0.01, 0.30)	0.032*	0.09 (−0.05, 0.23)	0.213
	>9:00	0.25 (0.05, 0.45)	0.015*	0.11 (−0.10, 0.31)	0.298
	Sleep midpoint				
	<3:30	Reference	/	Reference	/
	3:30–4:00	0.07 (−0.08, 0.22)	0.331	0.01 (−0.14, 0.16)	0.852
	>4:00	0.25 (0.12, 0.38)	0.001***	0.16 (0.03, 0.30)	0.017*

Notes: Adjusted model adjust for: age and BMI. The effect of wake up time and sleep midpoint on PCOS is expressed by OR and TT levels is expressed by β. *Indicated P < 0.05; **Indicated P<0.01; ***Indicated P<0.001.
Abbreviations: PCOS, polycystic ovary syndrome; BMI, body mass index; TT, total testosterone; OR, odds ratio.

compared to women with a sleep midpoint < 3:30. After adjusting for age OR and BMI, the risk of PCOS in women with a sleep midpoint > 4:00 continued to increase (OR = 2.39, 95% CI 1.22 to 4.70, P = 0.017). However, among women with BMI ≥ 24kg/cm^2, there was no such difference between women with different wake-up times and sleep midpoints.

Discussion

This study is the first to systematically use a two-sample MR approach to evaluate the causal relationships between getting up in the morning and self-reported chronotypes with PCOS. The effects of wake-up time and sleep midpoint on PCOS risk and TT levels were investigated in a cohort of women with PCOS and controls.

A person's natural inclination to go to bed at a specific time is known as their chronotype. When a person has an advanced or delayed sleep period, they are called "morningness" and "eveningness", respectively.[31] Our results show that the genetics of getting up in the morning and chronotype were negatively correlated with PCOS in the Finnish biobanks. A multicenter study indicated that genome-wide chronodisruption exists in the ovarian granulosa cells (GCs) of women with PCOS.[32] A previous study showed that polycystic ovary syndrome and metabolic dysregulation are linked to morning circadian dysrhythmia.[9] Johnson et al examined circadian rhythm genes in peripheral blood mononuclear cells (PBMCs) of women with PCOS and control participants. The results showed that PCOS patients had abnormal peripheral circadian rhythm gene expression and a significant decrease in core clock genes, while PBMCs with circadian locomotor output cycle kaput (CLOCK)/brain and muscle ARNT-like 1 (BMAL1) knockdown were able to regulate peripheral androgen metabolism.[16] Although the exact mechanism by which getting up in the morning and chronotype leads to an increase in PCOS is not well understood, our study suggests that circadian disruption at the genetic level may affect PCOS development.

To verify these results in a population, we examined the association between various wake-up times and sleep midpoints, and the occurrence of PCOS and TT levels in our cross-sectional study. Our data showed that non-PCOS women had earlier wake-up times and sleep midpoints than women with PCOS. In addition, our findings indicated that while later wake-up time and sleep midpoint were associated with an increased risk of PCOS and TT levels, only later sleep midpoint remained associated with an increased risk of PCOS and TT levels after adjusting for age and BMI. A recent observational study has shown that women with PCOS have a higher prevalence of the evening chronotype than the morning chronotype, which may lead to a worse hormonal and metabolic status.[10]

Following BMI stratification, an earlier wake-up time was linked to a reduced risk of PCOS and lower TT levels in women with BMI < 24 kg/m^2. However, a study conducted by Barrea et al found that individuals with PCOS with an evening chronotype exhibited a higher susceptibility to obesity and less regular exercise than those with morning chronotypes.[33] This suggests that a higher percentage of women with PCOS are in the evening chronotype and that these evening chronotype women have more serious metabolic disorders. Combined with our results, we suggest that independence of BMI, wake-up time, and sleep midpoint can also influence PCOS risk and TT levels. In a cross-sectional survey, patients with infertility had significantly poorer sleep quality and more nocturnal chronotypes, which may be related to the interaction between the ovarian cycle and chronotype.[34] This demonstrates that chronotype may influence the ovarian cycle, which may affect the onset and progression of PCOS. These results support our conclusion that the morning chronotype may be a protective factor in women with PCOS, especially in non-obese women with PCOS.

Overall, individuals with a genetic morning chronotype may have a lower risk of PCOS, but this does not exclude the influence of environmental and behavioral factors (eg, sleep quality, physical activity, or diet). A bidirectional MR study showed a bidirectional causal relationship between PCOS and an increased risk of obstructive sleep apnea syndrome (OSAS), which may affect sleep quality.[35] In previous studies, evening chronotype has been associated with the unhealthiest eating habits, and chronotype assessments can be an effective tool for screening the eating habits of women with PCOS.[33,36] Meanwhile, some concurrent conditions of PCOS have not been excluded, such as insulin resistance and Hashimoto thyroiditis.[37] Evening chronotype has been linked to the most severe insulin resistance in a prior observational cross-sectional study.[33] The results of an MR study reveal a notable cause-and-effect relationship between Getting up and Autoimmune hypothyroidism.[38] Future studies can further explore the mediation of these factors between morning chronotype and PCOS.

This study provided important results. To our knowledge, this is the first population-based causal study to thoroughly examine the link between getting up in the morning and chronotypes with PCOS. Our MR Analysis demonstrated that getting up in the morning and chronotype (morning person) affected the PCOS. These results were further validated in the population, where wake-up times and sleep midpoints were associated with a reduced risk of PCOS and reduced TT levels, and these results were more significant in women with a BMI of less than 24 kg/m^2.

However, our study has some limitations that cannot be ignored. First, stratified analyses, such as subgroups based on gender, age, income, and severity order, cannot be analyzed using GWAS data or our cross-sectional data. Consequently, the causal relationship between PCOS and sleep traits may have been imprecise without further stratification. Second, despite employing various methods to manage and evaluate pleiotropy, the inherent bias from gene pleiotropy cannot be completely eliminated. Third, the participants in our study only represented individuals of European ancestry, which would later need to be further expanded to include individuals of other ancestries. Fourth, the sleep conditions in this study were all self-reported, which could be further verified by objective measurements (eg, actigraphy). Fifth, an unbalanced case-control ratio may lead to selection bias and extrapolation of results. Finally, our study is a single-center cross-sectional cohort study that cannot establish causality and needs to be further validated by rigorous RCT studies.

Conclusion

In summary, our results highlight that getting up in the morning and chronotype (morning person) was associated with a lower risk of PCOS. Further cohort validation indicated that an earlier wake-up time (< 7:00) and an earlier sleep midpoint (< 3:30) reduced the risk of PCOS and lowered TT levels, with more significant effects observed in individuals with a BMI < 24 kg/m^2. Further mechanistic studies and clinical trials are required to validate these findings.

Data Sharing Statement

The UK Biobank [https://www.ukbiobank.ac.uk] and Finnish Biobank [https://www.finngen.fi/en/access_results] data are available to all researchers. Further data inquiries were obtained by contacting the corresponding authors.

Ethics Approval

The GWASs used in this study received ethical approval and informed consent for the initial study. All participants in the observational study signed an informed consent form after the Ethics Committee of Shanghai Tenth People's Hospital (SHSY-IEC-4.1/21-227/01) approved the study procedure.

Acknowledgments

We would like to acknowledge the UK Biobank data [https://www.ukbiobank.ac.uk] and Finnish Biobanks [https://www.finngen.fi/en/access_results] for providing GWAS summary data. We thank the CNSknowall platform (https://cnsknowall.com) for providing data analysis services. At the same time, we would like to acknowledge all the participants in this study.

Author Contributions

Diliqingna Dilimulati: Data curation, Investigation, Methodology, Project administration, Software, Visualization, Writing – original draft. Jiayi Lu: Data curation, Methodology, Project administration, Writing – original draft. Jinghua Li: Data curation, Methodology, Project administration, Writing – original draft. Meili Cai: Project administration, Writing – original draft. Yuqin Zhang: Project administration, Writing – original draft. Xiaowen Shao: Project administration, Writing – original draft. Haibing Chen: Project administration, Writing – original draft. Qian Wan: Project administration, Writing – original draft. Fang He: Project administration, Writing – original draft. Chaoyan Yue: Conceptualization, Formal analysis, Writing – original draft. Manna Zhang: Conceptualization, Supervision, Writing – review and editing. Shen Qu: Conceptualization, Funding acquisition, Writing – review and editing. All authors agreed on the journal to which the article will be submitted, reviewed and agreed on all versions of the article before submission, during revision, the final version accepted for publication, and any significant changes introduced at the proofing stage and agreed to take responsibility and be accountable for the contents of the article.

Funding

This research was supported by the National Natural Science Foundation of China (82170861), the Clinical Research Plan of SHDC (No. SHDC2020CR1017B), the Clinical Research Project of Shanghai National Health Commission (202340007), and Diabetes Metabolism Research of China International Medical Exchange Foundation (Z-2017-26-2202-4).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Teede HJ, Tay CT, Laven J, et al. Recommendations from the 2023 international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Fertil Steril*. 2023;120(4):767–793. doi:10.1016/j.fertnstert.2023.07.025
2. Azziz R, Carmina E, Chen Z, et al. Polycystic ovary syndrome. *Nat Rev Dis Primers*. 2016;2:16057. doi:10.1038/nrdp.2016.57
3. Cooney LG, Dokras A. Beyond fertility: polycystic ovary syndrome and long-term health. *Fertil Steril*. 2018;110(5):794–809. doi:10.1016/j.fertnstert.2018.08.021
4. Patel S. Polycystic ovary syndrome (PCOS), an inflammatory, systemic, lifestyle endocrinopathy. *J Steroid Biochem mol Biol*. 2018;182:27–36. doi:10.1016/j.jsbmb.2018.04.008
5. Escobar-Morreale HF. Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. *Nat Rev Endocrinol*. 2018;14(5):270–284. doi:10.1038/nrendo.2018.24
6. Bennett CJ, Mansfield DR, Mo L, et al. Sleep disturbances may influence lifestyle behaviours in women with self-reported polycystic ovary syndrome. *Br J Nutr*. 2021:1–9. doi:10.1017/S0007114521002361
7. Mo L, Mansfield DR, Joham A, et al. Sleep disturbances in women with and without polycystic ovary syndrome in an Australian national cohort. *Clin Endocrinol*. 2019;90(4):570–578. doi:10.1111/cen.13922
8. Moran LJ, March WA, Whitrow MJ, Giles LC, Davies MJ, Moore VM. Sleep disturbances in a community-based sample of women with polycystic ovary syndrome. *Hum Reprod*. 2015;30(2):466–472. doi:10.1093/humrep/deu318
9. Simon SL, McWhirter L, Diniz Behn C, et al. Morning circadian misalignment is associated with insulin resistance in girls with obesity and polycystic ovarian syndrome. *J Clin Endocrinol Metab*. 2019;104(8):3525–3534. doi:10.1210/jc.2018-02385

10. Barrea L, Verde L, Vetrani C, et al. Evening chronotype is associated with hormonal and metabolic disorders in polycystic ovary syndrome. *J Pineal Res*. 2023;74(2):e12844. doi:10.1111/jpi.12844
11. Tsereteli N, Vallat R, Fernandez-Tajes J, et al. Impact of insufficient sleep on dysregulated blood glucose control unDer standardised meal conditions. *Diabetologia*. 2022;65(2):356–365. doi:10.1007/s00125-021-05608-y
12. Facco FL, Grobman WA, Reid KJ, et al. Objectively measured short sleep duration and later sleep midpoint in pregnancy are associated with a higher risk of gestational diabetes. *Am J Obstet Gynecol*. 2017;217(447):e441–447e413. doi:10.1016/j.ajog.2017.05.066
13. Sellix MT, Menaker M. Circadian clocks in the ovary. *Trends Endocrinol Metab*. 2010;21(10):628–636. doi:10.1016/j.tem.2010.06.002
14. Chen M, Xu Y, Miao B, et al. Expression pattern of circadian genes and steroidogenesis-related genes after testosterone stimulation in the human ovary. *J Ovarian Res*. 2016;9(1):56. doi:10.1186/s13048-016-0264-5
15. Chen H, Isayama K, Kumazawa M, et al. Integration of the nuclear receptor REV-ERBα linked with circadian oscillators in the expressions of Alas1, Ppargc1a, and Il6 genes in rat granulosa cells. *Chronobiol Int*. 2015;32(6):739–749. doi:10.3109/07420528.2015.1042582
16. Johnson BS, Krishna MB, Padmanabhan RA, Pillai SM, Jayakrishnan K, Laloraya M. Derailed peripheral circadian genes in polycystic ovary syndrome patients alters peripheral conversion of androgens synthesis. *Hum Reprod*. 2022;37(8):1835–1855. doi:10.1093/humrep/deac139
17. Hu X, Zhao J, Lin Z, et al. Mendelian randomization for causal inference accounting for pleiotropy and sample structure using genome-wide summary statistics. *Proc Natl Acad Sci U S A*. 2022;119(28):e2106858119. doi:10.1073/pnas.2106858119
18. Gupta V, Walia GK, Sachdeva MP. "Mendelian randomization": an approach for exploring causal relations in epidemiology. *Public Health*. 2017;145:113–119. doi:10.1016/j.puhe.2016.12.033
19. Hemani G, Bowden J, Davey Smith G. Evaluating the potential role of pleiotropy in Mendelian randomization studies. *Hum Mol Genet*. 2018;27 (R2):R195–R208. doi:10.1093/hmg/ddy163
20. Gao X, Sun H, Zhang Y, Liu L, Wang J, Wang T. Investigating causal relations between sleep-related traits and risk of type 2 diabetes mellitus: a mendelian randomization study. *Front Genet*. 2020;11:607865. doi:10.3389/fgene.2020.607865
21. Jia Y, Guo D, Sun L, et al. Self-reported daytime napping, daytime sleepiness, and other sleep phenotypes in the development of cardiometabolic diseases: a Mendelian randomization study. *Eur J Prev Cardiol*. 2022;29(15):1982–1991. doi:10.1093/eurjpc/zwac123
22. 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. doi:10.1371/journal.pgen.1006125
23. Chen C, Lu FC. Department of disease control ministry of health PRC. The guidelines for prevention and control of overweight and obesity in Chinese adults. *Biomed Environ Sci*. 2004;17:1–36.
24. Rotterdam EA-SPCWG. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril*. 2004;81(1):19–25. doi:10.1016/j.fertnstert.2003.10.004
25. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res*. 1989;28(2):193–213. doi:10.1016/0165-1781(89)90047-4
26. Wang J, Li YR, Jiang CQ, et al. Chronotype and cognitive function: observational study and bidirectional Mendelian randomization. *EClinicalMedicine*. 2022;53:101713. doi:10.1016/j.eclinm.2022.101713
27. Zhao S, Zhao J, Wei S, Wang W, Wu Y, Yan B. Sleep timing and the prevalence of hypertension in middle-aged and older populations: the sleep heart health study. *BMC Psychiatry*. 2024;24(1):715. doi:10.1186/s12888-024-06174-4
28. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol*. 2015;44(2):512–525. doi:10.1093/ije/dyv080
29. Pagoni P, Dimou NL, Murphy N, Stergiakouli E. Using Mendelian randomisation to assess causality in observational studies. *Evid Based Ment Health*. 2019;22(2):67–71. doi:10.1136/ebmental-2019-300085
30. Greco MF, Minelli C, Sheehan NA, Thompson JR. Detecting pleiotropy in Mendelian randomisation studies with summary data and a continuous outcome. *Stat Med*. 2015;34(21):2926–2940. doi:10.1002/sim.6522
31. van der Merwe C, Munch M, Kruger R. Chronotype differences in body composition, dietary intake and eating behavior outcomes: a scoping systematic review. *Adv Nutr*. 2022;13(6):2357–2405. doi:10.1093/advances/nmac093
32. Wang F, Xie N, Wu Y, et al. Association between circadian rhythm disruption and polycystic ovary syndrome. *Fertil Steril*. 2021;115(3):771–781. doi:10.1016/j.fertnstert.2020.08.1425
33. Barrea L, Verde L, Vetrani C, Savastano S, Colao A, Muscogiuri G. Chronotype: a tool to screen eating habits in polycystic ovary syndrome? *Nutrients*. 2022;14(5):955. doi:10.3390/nu14050955
34. Ozcelik C, Varli B, Gokce A, Takmaz T, Cetin C, Ozcan P. Evaluation of chronotype and sleep quality in infertile population and comparison with fertile population: a cross-sectional study. *J Psychosom Obstet Gynaecol*. 2023;44(1):2148523. doi:10.1080/0167482X.2022.2148523
35. Liu P, Zhang Q, Ding H, Zou H. Association of obstructive sleep apnea syndrome with polycystic ovary syndrome through bidirectional Mendelian randomization. *Front Med*. 2024;11:1429783. doi:10.3389/fmed.2024.1429783
36. Kolnikaj TS, Herman R, Janez A, Jensterle M. Assessment of eating disorders and eating behavior to improve treatment outcomes in women with polycystic ovary syndrome. *Life*. 2022;13(1):12. doi:10.3390/life12111906
37. Gencer G, Serin AN, Gencer K. Analysis of the effect of hashimoto's thyroiditis and insulin resistance on ovarian volume in patients with polycystic ovary syndrome. *BMC Womens Health*. 2023;23(1):86. doi:10.1186/s12905-023-02200-x
38. Wang S, Wang K, Chen X, Lin S. The relationship between autoimmune thyroid disease, thyroid nodules and sleep traits: a mendelian randomization study. *Front Endocrinol*. 2023;14:1325538. doi:10.3389/fendo.2023.1325538