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ORIGINAL RESEARCH

Causal Relationship Between Sleep Traits and Hypothalamic-Pituitary-Target Gland Axis Function: A Mendelian Randomization Study

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Background: In recent years, multiple observational studies have confirmed the association between sleep traits and various human physiopathological states. However, the causal relationship between sleep traits and hypothalamic-pituitary-target gland axis (HPTGA) function remains unknown.

Methods: We obtained summary statistics on sleep traits (insomnia, chronotype, and sleep duration (long and short)) from the UK Biobank database. Data related to the HPTGA functions were obtained from the publicly available database. Subsequently, a two-sample Mendelian randomization (MR) analysis was performed to investigate the causal relationship between different sleep traits and the HPTGA function. Reverse MR analysis was conducted to examine the direction of causality.

Results: The MR analysis results suggested that chronotype is associated with decreased levels of six hormones in HPTGA. Sleep duration was causally associated with decreased levels of free thyroxine and progesterone. Both long and short sleep durations are detrimental to the secretion of prolactin-releasing peptide, somatostatin, and plasma cortisol, while short sleep duration can promote progesterone secretion. After gender stratification, we found that female reproductive function is more susceptible to the influence of unfavorable sleep traits.

Conclusion: Our MR analysis indicated a significant causal association between chronotype and suppressed gonadal function in healthy adult humans, with no apparent gender-specific effect. Extreme sleep durations were also found to be detrimental to the maintenance of normal HPTGA secretion function. Compared to males, gonadal function in the female cohort is more susceptible to extreme sleep habits. Subsequent observational studies are urgently needed to confirm the underlying mechanisms.

Keywords: hypothalamo-hypophyseal-target gland axis, sleep traits, causal inference, Mendelian randomization

Introduction

It is widely recognized in modern medicine that sleep is an active physiological process managed by the central nervous system, which specializes in sleep and wakefulness.[1] Sleep is essential not only for recovery from physical and mental illnesses but also for a range of neurological functions in the brain, such as neuronal cells and synaptic growth, and the construction of memory functions.[2] The hypothalamic-pituitary-target gland axis (HPTGA), as the most complex neuroendocrine regulatory system in the human body, participates in the regulation of a variety of endocrine metabolic processes by virtue of a sophisticated neurohumoral regulatory network. It plays a decisive role in maintaining homeostasis and regulating the function of endocrine organs.[3] There are numerous factors affecting the function of the hypothalamo-hypophyseal system,[4] but the association between sleep traits and their function is poorly understood and

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not systematic and is mostly based on observational studies. Whether there is a potential causal relationship between sleep traits and HPTGA function remains unknown.

There is a growing body of convincing evidence linking sleep disorders to the development of endocrine metabolic disorders.[5] There are also a few prospective observational studies suggesting that factors such as sleep rhythms, sleep time offsets, and other factors may be involved in influencing HPTGA function,[6] while some studies have taken the exact opposite view.[7] However, due to the extreme difficulty in obtaining relevant data, coupled with the likelihood that such designs may not fully account for confounding factors and reverse causality bias,[8] the causal relationship between sleep traits and HPTGA function remains unproven. To date, no study has explored the potential causal relationship between the two at the genetic level.

MR design is a genetic instrumental variable analysis method using observational data that can be employed to assess causal hypotheses between modifiable risk factors and outcomes such as disease.[9] This approach is less susceptible to measurement errors, confounding, and reverse causality compared to traditional multivariable regression methods, thereby significantly increasing the credibility of causal relationship analysis.[10] Therefore, it has been widely applied in recent years.

The aim of this study was to investigate whether there are causal associations of these factors on hypothalamic–pituitary–adrenal, thyroid and gonadal axis function using single-nucleotide polymorphism (SNP) data of genetic variants closely associated with sleep duration, sleep chronotype and insomnia symptoms from UK Biobank and IEU Open GWAS project databases. The primary and secondary relationships were also explored by bidirectional MR analysis tests.[11] Based on the aforementioned studies, we attempt to elucidate the role of sleep traits in maintaining the function of the hypothalamo-hypophyseal system and to provide a basis for future research on novel clinical treatment modalities and drug development.

Methods

Study Populations

The data on sleep traits used in this study were obtained from the UK Biobank database. In brief, over 500,000 UK residents were recruited by the UK Biobank research center between 2006 and 2010 in a prospective study of all people aged between 40 and 69 years, details of which can be found elsewhere.[12] A total of 503,325 participants (5.5%) were enrolled in the study cohort, and self-reported baseline data were collected via questionnaire and assessed with anthropometric indices. To avoid confounding effects and close relatives and affinities, individuals of non-White ethnicities (n=48,667, 0.53%) as well as participants' relatives (n=190,216, 2.1%) were excluded. The data on some relevant hormones and protein secretions of hypothalamic function (thyrotropin-releasing hormone (TRH), prolactin-releasing peptide (PrRP), corticotropin-releasing factor-binding protein (CRFBP), somatostatin (SRIF), gonadotropin-releasing hormone (GnRH)), pituitary function (growth hormone (GH), prolactin (PRL), luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), oxytocin-vasopressin 1), and thyroid function (thyroid peroxidase (TPO), thyroglobulin (Tg)) were obtained from the INTERVAL study conducted by England's National Health Service Blood and Transplant (NHSBT) from 2012 to 2014. The INTERVAL study included a total of 25 independent research centers and recruited approximately 50,000 blood donors. Participants completed an online questionnaire, which included information on demographic characteristics, anthropometric measurements, lifestyle, and dietary habits. Participants were generally in good health, and those with a history of major diseases (for example, hepatitis B or C, myocardial infarction, cancer, stroke, and AIDS) and those with a recent illness or infection were excluded from the cohort. For further SomaLogic testing, two nonoverlapping subgroups in the INTERVAL cohort were randomly selected, with one participant from each pair of close relatives (first- or second-degree) being excluded to eliminate consanguinity. After genetic quality control and sample quality control, including exclusion of sex mismatch, low call rates, duplicate samples, extreme heterozygosity, and non-European ancestry, 3301 participants were enrolled in subsequent plasma protein or protein complex concentration assays and genome-wide association analyses, as described elsewhere.[13]

Thyroid function-related circulating TSH and free thyroxine (FT4) data were obtained from a large-scale meta-analysis of a genome-wide association study on thyroid function and dysfunction. This analysis included TSH data from 54,288 participants in 22 independent cohorts and FT4 data from 49,269 participants in 19 cohorts. Only subjects with TSH levels within the normal reference range were included in the TSH and FT4 analyses. Individuals of non-European ancestry, on thyroid medication, or who had undergone previous thyroid surgery were excluded. Detailed descriptions of the quality control procedures can be found in.[14]

Data on the adrenal-related hormone plasma cortisol were obtained from a meta-analysis of a genome-wide association study (GWAS) of plasma cortisol comprising 11 Western European ethnic cohorts, including 12,579 subjects, from the CORtisol NETwork consortium. Inclusion criteria for study subjects were Caucasian adults 17 years of age or older; exclusion criteria were people on glucocorticoids, pregnant or lactating women, and twins (exclusion of one). A detailed description can be found in.[15]

Data related to gonadal hormones and related protein secretions were obtained from 2 different studies. E2, TT, BT, and sex hormone-binding globulin (SHBG) data were obtained from the UK Biobank Database. Self-identification by questionnaire as being of other than white European ancestry was excluded, as previously described. A maximum of 425,097 UK Biobank participants were available for analysis of genotypic and phenotypic data after application of quality control criteria (425,097 sera for analysis of SHBG, TT, and E2 levels and 382,988 sera for analysis of BT). Association tests were performed using linear mixed models implemented in BOLT-LMM to account for cryptic population structure and relatedness. Only those autosomal genetic variants that were common (minor allele frequency (MAF) >1%), passed quality control in all 106 batches and were present in both genotyping arrays were included in the genetic relationship matrix.[16]

Data related to progesterone (P4) and aldosterone (ALD) were obtained from the healthy adult cohort at the Leipzig Research Centre for Civilization Diseases research center in Germany. It included 10,000 citizens of Leipzig, Germany, from 2011–2016, and participants were phenotyped in detail for metabolic diseases and cognitive function, among other things. Samples were excluded from hormonal analysis if subjects were taking steroid medication, if quality control of the steroid profile indicated sample confounding, or if there was an underlying disease (eg, hypogonadotropic hypogonadism, androgen excess, congenital adrenal hyperplasia, adrenal insufficiency, or polycystic ovary syndrome), as described in detail here.[17]

Data on dehydroepiandrosterone (DHEA) sulfate were obtained from The United Kingdom Household Longitudinal Study, which surveyed 40,000 households in England, Scotland, Wales, and Northern Ireland. Participants have been surveyed annually since 2009, with computer-assisted interviews providing information about their socioeconomic status, attitudes, and behaviors. The study includes phenotypic data from a representative sample of participants across a wide range of social and economic indicators, as well as biospecimen collection, including biometric, physiological, biochemical, and blood measures, and self-reported medical history and medication use. A detailed description can be found elsewhere.[18]

Sleep Traits

All participants underwent a comprehensive questionnaire survey at baseline, where they were asked about their sleep chronotype (preference for morning or evening), average sleep duration, whether their sleep duration was long or short, and any symptoms of insomnia.

Insomnia Measure

At the baseline assessment, study participants self-reported their age, gender, symptoms of insomnia, and medication use using a touchscreen questionnaire. To assess insomnia symptoms, participants were asked, “Do you have difficulty falling asleep or waking up in the middle of the night?” with response options “never/rarely”, “sometimes”, “usually”, or “prefer not to answer”. Participants who responded “prefer not to answer” were defined as missing.

Chronotype Measure

The assessment of chronotype (morning or evening preference) requires participants to answer the question “Do you consider yourself to be?” with six possible response options: “definitely a ‘morning’ person”, “more of a ‘morning’ than

'evening' person", "more of an 'evening' than'morning' person", "definitely an 'evening' person", "do not know", or "prefer not to answer". These responses are encoded as 2, 1, −1, −2, 0, and missing, respectively.

Sleep Duration Measure

The measurement of sleep duration was assessed by asking participants: "On average, how many hours do you sleep in a 24-hour period? (Including naps)" with responses provided in hours. Sleep duration was treated as a continuous variable and was also categorized into short sleep duration (6 hours or less), normal sleep duration (7 or 8 hours), or long sleep duration (9 hours or more). Extreme responses of less than 3 hours or more than 18 hours were excluded, and responses of "do not know" or "prefer not to answer" were considered missing.

Exposure Data

The GWAS summary-level data on the genetic associations with sleep traits were obtained from three different self-reported studies conducted by the UK Biobank project between 2006 and 2010. In the subsequent MR analysis, we included 78 SNPs associated with continuous sleep duration, 26 SNPs associated with short sleep duration, and 10 SNPs associated with long sleep duration.[19] Additionally, we incorporated 41 SNPs related to insomnia symptoms[20] and 153 SNPs associated with chronotype.[21] For further details, please refer to Supplementary Table 1.

Outcome Data

In this study, three types of outcome variables were defined as hypothalamic function, pituitary function, and target gland function based on the physiological anatomical locations of the HPTGA. For hypothalamic function assessment, we measured the secretion levels of TRH, PrRP, CRFBP, SRIF, and GnRH. Pituitary function was assessed by GH, LH, FSH, ACTH, PRL, and oxytocin-neocortin 1 secretion levels. Cortisol, ALD, TSH, FT4, TPO, and Tg are used to assess adrenal and thyroid function. Due to physiological specificity, there are significant sex differences in male and female gonadal function. Therefore, in the present study, we used E2, P4, TT, BT, DHEA, and SHBG as indicators to assess gonadal function. We conducted analyses both within and across sexes (DHEA lacked sex-stratified data). The specific methods for sex stratification have been extensively described elsewhere.[22] We downloaded all feature data reported in the UK Biobank database (https://www.nealelab.is/uk-biobank), the IEU Open GWAS project (https://gwas.mrcieu.ac.uk/), the Thyroidomics Consortium (https://transfer.sysepi.medizin.uni-greifswald.de/thyroidomics/) and the Zenodo database (https://zenodo.org/). After excluding duplicate studies, malignancies, and non-European ancestry, we selected all GWAS summary-level data associated with hypothalamo-hypophyseal system functional assessment indicators, as detailed in Supplementary Table 2.

Instrumental Variable Selection

In the present study, sleep traits were used as the exposure, and HPTGA function served as the outcome (with the two being interchanged in a bidirectional MR analysis). To ensure the utmost authenticity and accuracy in assessing the causal relationship between sleep traits and HPTGA function, this study was conducted according to the following quality control criteria to select instrumental variables (IVs): ① SNPs associated with sleep traits with a significance threshold across the locus ($P<5.0\times10^{-8}$) were selected as potential IVs for the most precise analysis. ② The MAF threshold for variant SNPs of interest was 0.01, and SNPs with MAF≤0.01 were excluded. ③ One of the principles of MR is that the IVs included in the analysis should not be in strong linkage disequilibrium (LD) with each other, as the presence of strong LD may lead to biased results. LD between SNPs for each variant of interest was calculated according to the principles of 1000 Genomes project European samples for reference to ensure that R2<0.001 (clumping window size=10,000 kb), and only the SNPs with the lowest P values were retained. ④ To avoid distortion of strand orientation or allele coding, we deleted palindromic SNPs (eg, with A/T or G/C alleles). ⑤ During the harmonization process, we aligned the alleles with the human genome reference sequence (build 37) and removed ambiguous and duplicated SNPs to ensure the accuracy of the results.

Two-Sample MR Analysis

In this study, we conducted two-sample MR analyses between sleep traits and common hypothalamo-hypophyseal system hormones and protein secretions to explore causal associations. The six commonly used MR test methods were used for

characterization containing multiple IVs: maximum likelihood (ML) test, MR–Egger regression, simple median, weighted median, inverse variance weighted (IVW), and weighted model.[23–26] It has been reported that IVW, compared to the other five test methods, has the most stringent testing conditions and the most stable and powerful testing efficiency.[25] Therefore, in this study, the analysis method primarily relies on IVW results, with other test results used as supplementary evidence. We used the Benjamini-Hochberg method that controls the false discovery rate for multiple testing.[27]

Sensitivity Analyses

To further assess and correct potential violations of the MR assumptions in the obtained causal estimates, we performed heterogeneity testing using Cochran's Q statistics for the IVs that satisfy the significance level in at least one test method (IVW). Q statistics significant at a p value (Q-pval)<0.05 can imply the presence of heterogeneity.[28,29] MR–Egger regression is based on the assumption of instrument strength independent of direct effect, which makes it possible to evaluate the existence of pleiotropy with the intercept term. If the intercept term is equal to zero (MR–Egger regression P>0.05), this indicates that horizontal pleiotropy does not exist.[30] For IVs with heterogeneity, radial MR analysis was conducted using modified second-order weights to instrument the exposure to detect and remove outliers.[31] The new variants were subjected to reanalysis to minimize the impact of outliers on the overall analysis. In addition, to identify whether the causal outcomes were driven by a single SNP, "leave-one-out" analysis was performed by sequentially omitting each instrumental SNP.[32] Only results that met the above criteria were included in the final analysis. To circumvent the fact that reverse causal IVs will bias the MR estimate, we performed the MR Steiger directionality test to examine whether the exposure contributed to the outcome in a directional manner.[32] If Steiger_pval<0.05, it was taken to mean that the exposure only affected the outcome in a unidirectional way.

Bi-Directional MR Analysis

To explore whether HPTGA function has any causal effect on identified sleep traits, we also performed a reverse MR analysis (ie, hormone and protein secretions as exposure and identified sleep traits as outcome) using SNPs associated with HPTGA function as IVs to assess all potential reverse causality.

All analyses were performed using R 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria). Two-sample MR analyses were conducted using the "TwoSampleMR"[33] and "RadialMR" R package.[31]

Results

SNP Selection

Based on the aforementioned IV selection criteria and excluding linkage disequilibrium using a 1000-genome reference panel (R2<0.01 and clumping distance=10,000 kb), we identified 41 IVs associated with insomnia, 153 IVs associated with chronotype, 78 IVs associated with sleep duration, 26 IVs associated with short sleep duration, and 10 IVs associated with long sleep duration. Detailed information regarding the selected instrumental variables can be found in Supplementary Table 1. All F-statistic values of all IVs are greater than 10, indicating that there was no significant weak instrumental bias.

We performed MR testing on sleep trait data containing multiple SNPs using six fundamental MR methods. Sensitivity analysis was conducted on the results that had an IVW p value<0.05 based on the aforementioned methods. All analysis results showing heterogeneity were subjected to the radial MR test. Finally, after removing outliers, the data were included in the final analysis.

Causal Effects of Chronotype on the Negative Regulation of Hypothalamic, Pituitary, and Target Gland Functions

As shown in Table 1, Figure 1, Supplementary Figures and Supplementary Tables 3–7, after polytropy correction and outlier removal, at least one MR testing method indicates that chronotype is the only factor among sleep traits that can modulate the function of the tertiary regulatory centers of the HPTGA separately. After IVW assessment, we found that chronotype is causally associated with the secretion of CRFBP in the hypothalamus (beta value=−0.25, 95% CI=−0.47 to −0.03, P=0.03, Q-P value=0.296), the secretion of FSH (beta value=−0.22, 95% CI=−0.45 to 0.01, P=0.050,

Table 1 MR Estimation of Causal Associations Between Sleep Traits and Hypothalamo-Hypophyseal-Target Gland Axis Function

Exposure	Outcome	MR Methods	No. of SNP	β	lo_95% CI	up_95% CI	P-value	Q-pval	Steiger-pval	Correct Causal Direction
Short sleep	PrRP	Maximum likelihood	24	−1.899146	−3.603258	−0.19503447	0.02893869	0.7531616	0.5302767	TRUE
		MR Egger	24	4.6157694	−3.287855	12.5193938	0.26465393			
		Simple median	24	−2.3324707	−4.675104	0.01016255	0.05099791			
		Weighted median	24	−0.9160386	−3.328752	1.49667496	0.45678201			
		Inverse variance weighted	24	−1.9545228	−3.637117	−0.27192873	0.0228005			
		Weighted mode	24	0.6725403	−4.544513	5.88959368	0.80276975			
Long sleep	PrRP	Maximum likelihood	8	−4.823441	−9.222075	−0.424807	0.03161134	0.6197573	0.02554583	TRUE
		MR Egger	8	−4.898108	−17.99487	8.19865	0.49117599			
		Simple median	8	−5.120672	−11.20822	0.966873	0.11705592			
		Weighted median	8	−2.736716	−8.506342	3.032911	0.3593918			
		Inverse variance weighted	8	−4.726278	−9.038174	−0.414382	0.03168517			
		Weighted mode	8	−2.970338	−10.2797	4.339025	0.43194594			
Chronotype	CRFBP	Maximum likelihood	146	−0.2475692	−0.466149	−0.02898929	0.02642242	0.2962619	<0.001	TRUE
		MR Egger	146	−0.4353058	−1.120674	0.25006197	0.21519867			
		Simple median	146	−0.2583349	−0.576041	0.05937126	0.11832664			
		Weighted median	146	−0.2654122	−0.597622	0.06679789	0.11694828			
		Inverse variance weighted	146	−0.2450328	−0.4674	−0.02266581	0.03078953			
		Weighted mode	146	−0.4959749	−1.359726	0.36777612	0.29151923			
Long sleep	SRIF	Maximum likelihood	8	−5.667209	−10.07735	−1.2570732	0.0117795	0.8221315	0.02796002	TRUE
		MR Egger	8	−12.456992	−25.54784	0.6338551	0.11143359			
		Simple median	8	−6.073374	−11.79844	−0.348307	0.03911455			
		Weighted median	8	−5.724122	−11.38927	−0.0589709	0.04765833			
		Inverse variance weighted	8	−5.613575	−9.926155	−1.3009955	0.01073274			
		Weighted mode	8	−5.770263	−12.65021	1.1096877	0.13103776			
Sleep duration	FT4	Maximum likelihood	39	−0.003746815	−0.00631	−1.18E-03	0.004168562	0.4558611	<0.001	TRUE
		MR Egger	39	−0.006941392	−0.018453	4.57E-03	0.244804027			
		Simple median	39	−0.003651685	−0.007521	2.18E-04	0.064352147			
		Weighted median	39	−0.003655062	−0.007261	−4.94E-05	0.046936122			
		Inverse variance weighted	39	−0.003724806	−0.006268	−1.18E-03	0.004098102			
		Weighted mode	39	0.003323977	−0.005584	1.22E-02	0.469039815			

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Long sleep	TPO	Maximum likelihood	8	−5.338876	−9.755583	−0.9221694	0.01782513	0.3416165	0.08316805	TRUE
		MR Egger	8	3.039292	−10.25643	16.3350158	0.66984846			
		Simple median	8	−6.409133	−12.64739	−0.1708776	0.04404229			
		Weighted median	8	−5.219895	−11.1084	0.6686083	0.08230735			
		Inverse variance weighted	8	−5.205568	−9.78661	−0.6245258	0.02593368			
		Weighted mode	8	−3.512757	−11.43521	4.409695	0.41362227			
Chronotype	Plasma cortisol	Maximum likelihood	62	−0.2207635	−0.397883	−0.04364421	0.0145671	0.1128979	0.4365012	FALSE
		MR Egger	62	−0.7172232	−1.358166	−0.07628007	0.03217503			
		Simple median	62	−0.1776841	−0.434658	0.07928921	0.19719786			
		Weighted median	62	−0.1729567	−0.429988	0.08407416	0.199798			
		Inverse variance weighted	62	−0.2166846	−0.410016	−0.02335364	0.02803756			
		Weighted mode	62	−0.1609874	−0.638596	0.31662118	0.52533509			
Short sleep	Plasma cortisol	Maximum likelihood	13	−1.425964	−2.626342	−0.22558623	0.01989397	0.5959645	<0.001	TRUE
		MR Egger	13	0.7637364	−5.119942	6.64741476	0.80386482			
		Simple median	13	−1.6658117	−3.369566	0.03794298	0.04794556			
		Weighted median	13	−1.6455935	−3.215101	−0.07608565	0.04906724			
		Inverse variance weighted	13	−1.4430481	−2.626589	−0.25950699	0.01685958			
		Weighted mode	13	−1.6283951	−4.28097	1.02418017	0.2564192			
Sleep duration	P4	Maximum likelihood	73	−0.003756578	−0.007388	−0.00012532	0.04259697	0.3033175	<0.001	TRUE
		MR Egger	73	−0.006114949	−0.020305	0.008075052	0.40115463			
		Simple median	73	−0.002572424	−0.008073	0.002928463	0.35936766			
		Weighted median	73	−0.001499494	−0.006973	0.003974004	0.59130042			
		Inverse variance weighted	73	−0.00373919	−0.007466	−1.1902E-05	0.04926876			
		Weighted mode	73	−0.000969822	−0.010812	0.008872459	0.84739999			
Short sleep	P4	Maximum likelihood	24	1.1018929	0.1912548	2.012531	0.0177091	0.5720261	0.9564324	TRUE
		MR Egger	24	0.9695932	−3.23027	5.169456	0.65534533			
		Simple median	24	0.6629186	−0.60048	1.926317	0.30374631			
		Weighted median	24	0.545556	−0.779263	1.870375	0.41959646			
		Inverse variance weighted	24	1.0580915	0.161611	1.954572	0.02070425			
		Weighted mode	24	0.2686674	−2.302913	2.840248	0.83955149			
Chronotype	DHEA-sulphate	Maximum likelihood	66	−0.1463097	−0.258454	−0.03416585	0.010553809	0.09756528	0.3208448	FALSE
		MR Egger	66	−0.2701208	−0.608886	0.06864401	0.123020786			
		Simple median	66	−0.3004826	−0.474756	−0.12620961	0.000726324			
		Weighted median	66	−0.257487	−0.436871	−0.07810275	0.004902497			
		Inverse variance weighted	66	−0.1433748	−0.266336	−0.02041348	0.022289957			
		Weighted mode	66	−0.4540321	−0.872123	−0.03594091	0.037091888			

(*Continued*)

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Table 1 (Continued).

Exposure	Outcome	MR Methods	No. of SNP	β	lo_95% CI	up_95% CI	P-value	Q-pval	Steiger-pval	Correct Causal Direction
Chronotype	TT	Maximum likelihood	116	−0.03267482	−0.045464	−0.01988552	5.51367E-07	0.8961943	<0.001	TRUE
		MR Egger	116	−0.04713691	−0.084758	−0.00951612	0.01556704			
		Simple median	116	−0.03405433	−0.052775	−0.01533414	0.00036319			
		Weighted median	116	−0.03353506	−0.05296	−0.01411057	0.000714882			
		Inverse variance weighted	116	−0.03274512	−0.045393	−0.02009696	3.88945E-07			
		Weighted mode	116	−0.04783772	−0.094475	−0.00120081	0.04672182			
Chronotype	BT	Maximum likelihood	113	−0.06704449	−0.087087	−0.04700196	5.51153E-11	0.5044926	<0.001	TRUE
		MR Egger	113	−0.08962342	−0.14682	−0.032427	0.002681427			
		Simple median	113	−0.05656518	−0.086435	−0.02669532	0.000205878			
		Weighted median	113	−0.05947425	−0.090864	−0.02808427	0.00020434			
		Inverse variance weighted	113	−0.06700474	−0.086766	−0.04724375	3.01401E-11			
		Weighted mode	113	−0.04421704	−0.116705	0.02827085	0.234384			

Note: The red font that appears in the table indicates statistical significance for the analysis results ($P < 0.05$).

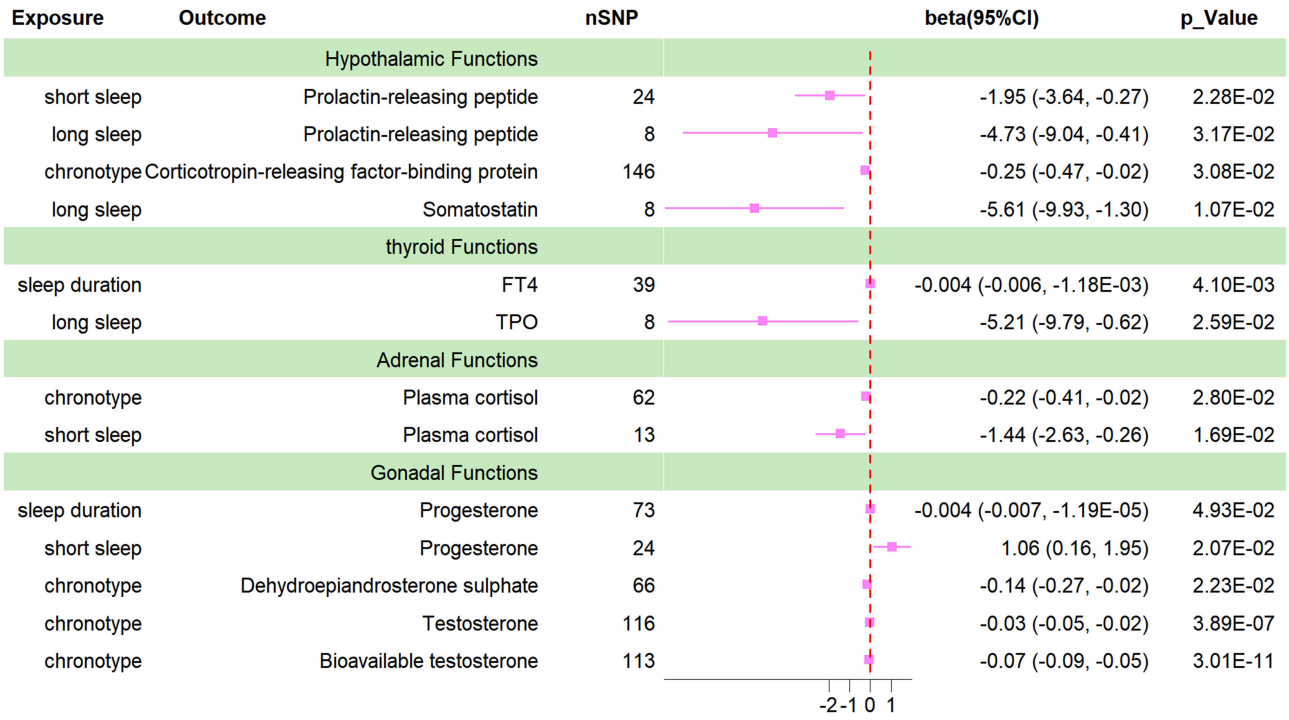

Figure 1 Forrest plot for the causal association between sleep traits and HPTGA.

Q-P value=0.219) and PRL (beta value=−0.49, 95% CI=−0.79 to −0.19, P=0.001, Q-P value=0.629) in the pituitary, adrenal cortisol levels (beta value=−0.22, 95% CI=−0.41 to −0.02, P=0.028, Q-P value=0.113), gonadal (not differentiated by sex) DHEA sulfate levels (beta value=−0.14, 95% CI=−0.27 to −0.02, P=0.022, Q-P value=0.098), TT levels (beta value=−0.03, 95% CI=−0.05 to −0.02, P=3.89E-07, Q-P value=0.896), and bioavailable testosterone (BT) levels (beta value=−0.07, 95% CI=−0.09 to −0.05, P=3.01E-11, Q-P value=0.504).

Causal Effects of Sleep Duration on the Negative Regulation of Hypothalamic and Target Gland Functions

As shown in Table 1, Figure 1, Supplementary Figures and Supplementary Tables 3–7, both long sleep and short sleep are causally associated with hypothalamic PrRP secretion. Sleep duration has a causal relationship with thyroid FT4 secretion, and long sleep has a causal relationship with thyroid TPO secretion. Short sleep is causally associated with adrenal cortisol secretion. Both sleep duration and short sleep duration have causal relationships with P4 secretion. Interestingly, short sleep appears to be beneficial for promoting P4 secretion. Sleep duration also has a causal relationship with DHEA secretion. However, after observation, we did not find any evidence supporting a causal effect of sleep duration on pituitary function.

Female Gonadal Function is More Susceptible to Regulation and Influence by Sleep Trait-Related Genotypic Variants

Due to the significant differences in gonadal function among different gender groups, we further stratified the sex hormone secretion data by gender. As seen in Table 2, Figure 2, and Supplementary Table 8, there was a significant causal effect of chronotype on TT (beta value=−0.07, 95% CI=−0.10 to −0.04, P=5.15E-06, Q-P value=0.524) and BT (beta value=−0.06, 95% CI=−0.09 to −0.03, P=1.37E-04, Q-P value=0.522) secretion in males. Female participants showed even more pronounced effects, with significant causal effects of chronotype not only on TT (beta value=−0.05, 95% CI=−0.08 to −0.03, P=1.60E-04, Q-P value=0.701) and BT (beta value=−0.07, 95% CI=−0.09 to −0.05, P=2.18E-08, Q-P value=0.653) levels in females but also exhibited a causal association with estrogen secretion (beta value=−0.12,

Table 2 MR Estimation of Causal Associations Between Sleep Traits and Gonadal Function (Stratified by Gender)

Exposure	Outcome	MR Methods	No. of SNP	β	lo_95% CI	Up_95% CI	P-value	Q_pval	Steiger_pval	Correct Causal Direction
Chronotype	TT (Male)	Maximum likelihood	115	−0.06972461	−0.1000461	−0.03940308	6.5739E-06	0.5243261	<0.001	TRUE
		MR Egger	115	−0.03391672	−0.1197769	0.05194341	0.4404029			
		Simple median	115	−0.0825231	−0.1304015	−0.03464471	0.00072948			
		Weighted median	115	−0.06591712	−0.1125596	−0.01927464	0.00560641			
		Inverse variance weighted	115	−0.06968647	−0.0996494	−0.03972355	5.1524E-06			
		Weighted mode	115	0.03880046	−0.1159547	0.19355565	0.6240783			
Chronotype	BT (Male)	Maximum likelihood	121	−0.05657817	−0.08612065	−0.027035696	0.00017425	0.5217495	<0.001	TRUE
		MR Egger	121	−0.08412406	−0.1684376	0.000189479	0.05285666			
		Simple median	121	−0.04789414	−0.09010296	−0.005685312	0.02614831			
		Weighted median	121	−0.05489468	−0.09779847	−0.011990886	0.01214899			
		Inverse variance weighted	121	−0.05683917	−0.08604762	−0.027630718	0.00013666			
		Weighted mode	121	−0.04302881	−0.14964928	0.063591664	0.43050637			
Chronotype	E2 (Female)	Maximum likelihood	121	−0.120998638	−0.236846	−0.005151292	0.04064299	0.3165679	<0.001	TRUE
		MR Egger	121	−0.384823283	−0.7398531	−0.029793478	0.03569931			
		Simple median	121	−0.008074901	−0.1831688	0.167018988	0.92646295			
		Weighted median	121	−0.030894921	−0.2073956	0.145605771	0.72518333			
		Inverse variance weighted	121	−0.119875322	−0.2376392	−0.002111469	0.04602737			
		Weighted mode	121	0.061485928	−0.3409723	0.463944161	0.77404898			
Chronotype	TT (Female)	Maximum likelihood	115	−0.05478464	−0.08365743	−0.025911848	0.00020001	0.701352	<0.001	TRUE
		MR Egger	115	−0.0272778	−0.1259152	0.071359604	0.58886475			
		Simple median	115	−0.04176238	−0.0822543	−0.001270468	0.04322838			
		Weighted median	115	−0.04296322	−0.08480631	−0.001120134	0.04417053			
		Inverse variance weighted	115	−0.05495087	−0.08348665	−0.02641508	0.00016043			
		Weighted mode	115	−0.03151171	−0.12919519	0.066171779	0.52847246			
Chronotype	BT (Female)	Maximum likelihood	119	−0.0697034	−0.09446845	−0.04493834	3.4564E-08	0.6526455	<0.001	TRUE
		MR Egger	119	−0.09892209	−0.17138455	−0.02645963	0.0085268			
		Simple median	119	−0.0846103	−0.11993993	−0.04928066	2.6796E-06			
		Weighted median	119	−0.08673794	−0.12245326	−0.05102262	1.9355E-06			
		Inverse variance weighted	119	−0.06984823	−0.09430962	−0.04538683	2.185E-08			
		Weighted mode	119	−0.11450155	−0.1978315	−0.0311716	0.00810807			

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Sleep duration	BT (Female)	Maximum likelihood	57	0.001012833	2.51E-04	0.001774882	0.00918683	0.6830147	<0.001	TRUE
		MR Egger	57	0.003080642	3.32E-04	0.005829296	0.03227187			
		Simple median	57	0.001470245	3.97E-04	0.002543191	0.00723644			
		Weighted median	57	0.001545493	3.77E-04	0.002714352	0.0095542			
		Inverse variance weighted	57	0.001009217	2.54E-04	0.001764385	0.00880918			
		Weighted mode	57	0.002250903	7.17E-06	0.004494633	0.05423211			

Note: The red font that appears in the table indicates statistical significance for the analysis results ($P < 0.05$).

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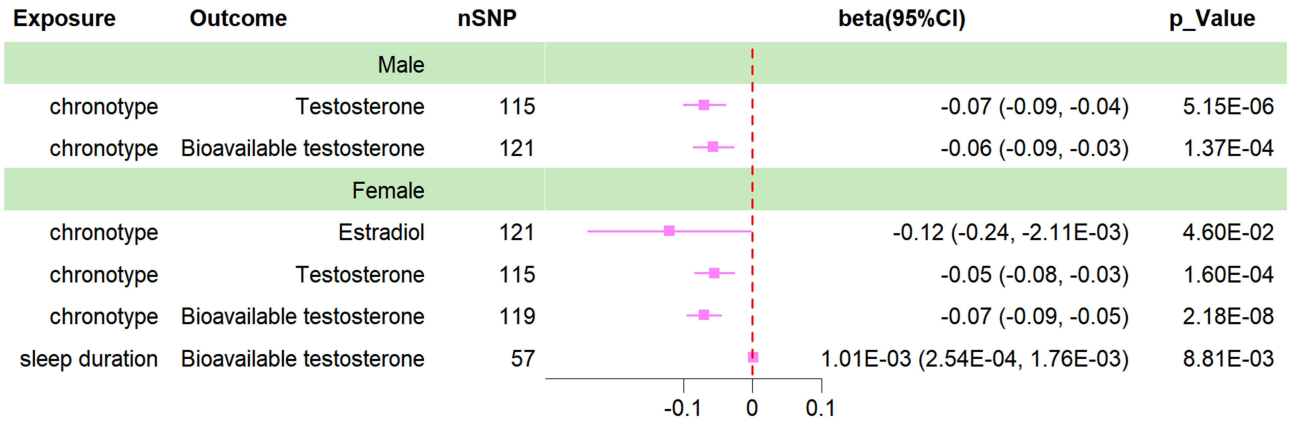

Figure 2 Forrest plot for the causal association between sleep traits and gonadal function (stratified by gender).

95% CI=−0.24 to −0.002, P=4.60E-02, Q-P value=0.316). Furthermore, there was also a significant causal association between sleep duration and BT secretion in females (beta value=0.001, 95% CI=2.54E-04 to 1.76E-03, P=8.81E-03, Q-P value=0.683). In Supplementary Table 8, it can be observed that sleep duration, short sleep, and long sleep traits all show certain trends of impact on the secretion of P4, TT, and SHBG in the female cohort. However, unfortunately, only the ML test yielded a p value of less than 0.05, failing to meet the required statistical significance threshold for this study.

Insomnia Does Not Modulate HPTGA Functions

As shown in the Supplemental Table, there is no causal relationship between HPTGA function and genetic susceptibility related to insomnia.

Bidirectional Causal Effects Between HPTGA Function and Sleep Traits

To assess any potential reverse causality effects that may exist, we performed reverse MR analyses using all hypothalamo-hypophyseal system functions for which a causal association existed as exposure and sleep traits as outcome, as detailed in Table 4. We found a bidirectional causal effect between circulating TT (OR=0.97, 95% CI=0.95 to 0.99, P=7.67E-03), BT levels (OR=0.95, 95% CI=0.92 to 0.98, P=3.65E-03) and chronotype in women. Similarly, there was a bidirectional causal effect between BT and sleep duration (OR=1.03, 95% CI=1.01 to 1.05, P=1.62E-02) in females. The above phenomena were observed only in the female cohort, and no reverse causal association was observed between the hypothalamo-hypophyseal system and sleep traits in the male cohort. See Tables 3 and 4 for details.

Discussion

To the best of our knowledge, the present study is the first investigation using MR analysis to examine the causal relationship between sleep traits and HPTGA function at the genetic level. Based on comprehensive genomic data from over 400,000 European participants, we compared multiple exposure factors (chronotype, insomnia, long sleep, short sleep, and sleep duration) to infer potential causal associations between sleep traits and HPTGA function. Strong evidence suggests that the morning chronotype is associated with decreased circulating TT and BT levels. Extreme sleep durations (either too long or too short) are detrimental to the production and secretion of multiple hormones in the hypothalamo-hypophyseal system.

The hypothalamic-pituitary system comprises three main regulatory axes: the hypothalamic-pituitary-ovarian (HPO) axis, hypothalamic-pituitary-thyroid (HPT) axis, and hypothalamic–pituitary–adrenal (HPA) axis. The hypothalamus and pituitary gland, as the highest-level endocrine regulatory centers in the body, are responsible for producing various proteins and peptide fragments to modulate the secretion of hormones in downstream target organs.[34] Previous studies have suggested that changes in sleep duration and circadian rhythms can influence hormone levels through humoral and neural pathways. For example, numerous cellular oscillators in the suprachiasmatic nucleus of the hypothalamus are

Table 3 Bi-Directional MR Estimation of the Causal Effects Between Hypothalamic-Pituitary System Function and Sleep Traits

Exposure (Thalamo-Pituitary Axis Function)	Outcome (Sleep Traits)	MR Methods	No. of SNP	OR	lo_95% CI	Up_95% CI	P-value
PrRP	Short sleep	Maximum likelihood	3	0.9987461	0.9898189	1.007754	0.7841667
		MR Egger	3	1.0160767	0.9570099	1.078789	0.6937526
		Simple median	3	1.0005278	0.9895579	1.011619	0.9252642
		Weighted median	3	1.0002122	0.9893568	1.011187	0.9696024
		Inverse variance weighted	3	0.9987516	0.9898483	1.007735	0.7845314
		Weighted mode	3	1.0006889	0.9882896	1.013244	0.9236736
PrRP	Long sleep	Maximum likelihood	3	1.0039558	0.9970556	1.010904	0.2618652
		MR Egger	3	0.9799676	0.9364789	1.025476	0.542826
		Simple median	3	1.0013267	0.9926593	1.01007	0.7650155
		Weighted median	3	1.0013211	0.9921362	1.010591	0.7788664
		Inverse variance weighted	3	1.0038955	0.9971085	1.010729	0.2612888
		Weighted mode	3	1.0011251	0.9910015	1.011352	0.848444
CRFBP	Chronotype	Maximum likelihood	4	1.003153	0.9955674	1.010797	0.4162498
		MR Egger	4	0.999969	0.9850131	1.015152	0.9971484
		Simple median	4	1.005796	0.9894698	1.022391	0.4888615
		Weighted median	4	1.002738	0.9949929	1.010544	0.4894318
		Inverse variance weighted	4	1.003152	0.99557	1.010792	0.4161873
		Weighted mode	4	1.002628	0.9953678	1.009941	0.5300779
SRIF	Long sleep	Maximum likelihood	5	1.0022044	0.9973202	1.007113	0.3770068
		MR Egger	5	0.9928414	0.9607473	1.026008	0.6971697
		Simple median	5	1.0012235	0.9949451	1.007542	0.7032092
		Weighted median	5	1.000765	0.9948504	1.006715	0.8003837
		Inverse variance weighted	5	1.0021707	0.9973343	1.007031	0.3796533
		Weighted mode	5	0.9996751	0.9918165	1.007596	0.9395648
FT4	Sleep duration	Maximum likelihood	22	1.0012716	0.9837059	1.019151	0.88808823
		MR Egger	22	0.9383519	0.8732612	1.008294	0.09816261
		Simple median	22	0.999449	0.9722247	1.027436	0.9687994
		Weighted median	22	0.995096	0.968645	1.022269	0.72060663
		Inverse variance weighted	22	1.0012382	0.9736835	1.029573	0.93074443
		Weighted mode	22	0.9895829	0.9566205	1.023681	0.55110027
TPO	Long sleep	Maximum likelihood	4	1.002556	0.9967781	1.008366	0.3867225
		MR Egger	4	1.002047	0.9886251	1.015652	0.7943025
		Simple median	4	1.001999	0.9950762	1.00897	0.5723859
		Weighted median	4	1.00315	0.9962755	1.010072	0.3700283
		Inverse variance weighted	4	1.002525	0.9967844	1.008299	0.389386

(*Continued*)

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Table 3 (Continued).

Exposure (Thalamo-Pituitary Axis Function)	Outcome (Sleep Traits)	MR Methods	No. of SNP	OR	lo_95% CI	Up_95% CI	P-value
		Weighted mode	4	1.003631	0.9951823	1.012152	0.4624206
Plasma cortisol	Chronotype	Maximum likelihood	4	0.9950271	0.9753313	1.015121	0.6250234
		MR Egger	4	0.9995786	0.9302549	1.074068	0.9918726
		Simple median	4	1.0053052	0.9790907	1.032222	0.6946861
		Weighted median	4	1.0002195	0.9767892	1.024212	0.9855206
		Inverse variance weighted	4	0.9951855	0.9641964	1.027171	0.7649265
		Weighted mode	4	0.9932686	0.9718005	1.015211	0.587385
Plasma cortisol	Short sleep	Maximum likelihood	4	1.000816	0.993804	1.007878	0.8201188
		MR Egger	4	0.9989501	0.9859728	1.012098	0.8893515
		Simple median	4	1.0020582	0.9920696	1.012147	0.6874903
		Weighted median	4	1.0022161	0.9940702	1.010429	0.5949804
		Inverse variance weighted	4	1.0008118	0.9938128	1.00786	0.8207155
		Weighted mode	4	1.0022706	0.9935772	1.01104	0.6450339
P4	Sleep duration	Maximum likelihood	5	1.019139	0.9924249	1.046571	0.16184236
		MR Egger	5	1.062437	1.0116354	1.115791	0.09393702
		Simple median	5	1.004104	0.9624167	1.047597	0.84984615
		Weighted median	5	1.015164	0.9790411	1.052619	0.41556104
		Inverse variance weighted	5	1.018121	0.9781486	1.059726	0.37950092
		Weighted mode	5	1.005514	0.9608197	1.052288	0.82426422
P4	Short sleep	Maximum likelihood	5	0.9952397	0.984377	1.006222	0.3941073
		MR Egger	5	0.9911876	0.9715402	1.011232	0.4499488
		Simple median	5	0.9879256	0.9733402	1.00273	0.1094232
		Weighted median	5	0.9908298	0.9773948	1.00445	0.1859649
		Inverse variance weighted	5	0.9953435	0.9846257	1.006178	0.3981215
		Weighted mode	5	0.9878879	0.9714243	1.00463	0.2283014
DHEA-sulphate	Chronotype	Maximum likelihood	7	0.9928568	0.9627972	1.023855	0.6476483
		MR Egger	7	0.9539083	0.9113098	0.998498	0.0988033
		Simple median	7	1.0389116	0.9806824	1.100598	0.1945773
		Weighted median	7	0.9781299	0.9435486	1.013979	0.2285526
		Inverse variance weighted	7	0.9929891	0.9611874	1.025843	0.6718237
		Weighted mode	7	0.9680047	0.9332975	1.004003	0.131494
TT	Chronotype	Maximum likelihood	218	0.9245942	0.8939888	0.9562474	5.00E-06
		MR Egger	218	0.9746326	0.8870323	1.070884	5.93E-01
		Simple median	218	0.9455388	0.8886737	1.0060426	7.68E-02
		Weighted median	218	0.9838269	0.9289919	1.0418986	5.77E-01

BT	Chronotype	Inverse variance weighted	218	0.9244457	0.8770543	0.974398	3.43E-03
		Weighted mode	218	0.9601648	0.8932942	1.0320412	2.71E-01
		Maximum likelihood	154	0.9814087	0.9543583	1.009226	0.1881759
		MR Egger	154	0.9536476	0.8771526	1.036814	0.2676587
		Simple median	154	0.9838948	0.9392938	1.030614	0.4927235
		Weighted median	154	0.9840045	0.9342489	1.03641	0.5424594
		Inverse variance weighted	154	0.9819652	0.9416689	1.023986	0.3946097
		Weighted mode	154	0.9711541	0.9183106	1.027038	0.3068024

Note: The red font that appears in the table indicates statistical significance for the analysis results ($P < 0.05$).

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Table 4 Bi-Directional MR Estimation of the Causal Effects Between Gonadal Function and Sleep Traits (Stratified by Gender)

Exposure (Thalamo-Pituitary Axis Function)	Outcome (Sleep Traits)	MR Methods	No. of SNP	OR	lo_95% CI	Up_95% CI	P-value
TT	Chronotype (Male)	Maximum likelihood	208	1.0014666	0.9877552	1.015368	0.8349462
		MR Egger	208	1.0123555	0.9696498	1.056942	0.5771562
		Simple median	208	0.9970741	0.9711706	1.023669	0.8272888
		Weighted median	208	0.9968513	0.9702438	1.024188	0.8192776
		Inverse variance weighted	208	1.0014531	0.976287	1.027268	0.9109645
		Weighted mode	208	1.0020812	0.9779265	1.026833	0.867529
BT	Chronotype (Male)	Maximum likelihood	109	0.990263	0.971203	1.009697	0.3237499
		MR Egger	109	1.0159862	0.950798	1.085644	0.6401937
		Simple median	109	0.9902011	0.9563692	1.02523	0.578768
		Weighted median	109	0.9942141	0.9581592	1.031626	0.7581632
		Inverse variance weighted	109	0.9906581	0.9538689	1.028866	0.6268832
		Weighted mode	109	0.9970363	0.9635948	1.031638	0.8649189
E2	Chronotype (Female)	Maximum likelihood	10	0.9962145	0.9700705	1.023063	0.7798388
		MR Egger	10	0.9615864	0.8926802	1.035811	0.3320252
		Simple median	10	0.9823719	0.9463031	1.019815	0.351393
		Weighted median	10	0.9815298	0.9449055	1.019574	0.3366073
		Inverse variance weighted	10	0.9963021	0.9617757	1.032068	0.8368846
		Weighted mode	10	0.9709655	0.9105736	1.035363	0.3919141
TT	Chronotype (Female)	Maximum likelihood	246	0.9700781	0.9563419	0.9840116	2.98E-05
		MR Egger	246	0.9528544	0.9120446	0.9954902	3.16E-02
		Simple median	246	0.9785467	0.9544844	1.0032155	8.78E-02
		Weighted median	246	0.9779916	0.9501006	1.0067014	1.32E-01
		Inverse variance weighted	246	0.9700718	0.9486441	0.9919835	7.67E-03
		Weighted mode	246	0.9811323	0.9460072	1.0175615	3.07E-01
BT	Chronotype (Female)	Maximum likelihood	179	0.9512116	0.9320084	0.9708105	1.53E-06
		MR Egger	179	0.9598556	0.8978559	1.0261366	2.31E-01
		Simple median	179	0.9564012	0.9225337	0.9915121	1.54E-02
		Weighted median	179	0.9829208	0.9452296	1.022115	3.88E-01
		Inverse variance weighted	179	0.9509987	0.9193241	0.9837646	3.65E-03
		Weighted mode	179	0.9705716	0.9305674	1.0122956	1.66E-01
BT	Sleep duration (Female)	Maximum likelihood	179	1.02982	1.012431	1.047509	7.20E-04
		MR Egger	179	1.027148	0.9807259	1.075767	2.58E-01
		Simple median	179	1.042219	1.0115288	1.073841	6.69E-03
		Weighted median	179	1.026838	0.9965426	1.058054	8.30E-02
		Inverse variance weighted	179	1.029193	1.0053297	1.053623	1.62E-02
		Weighted mode	179	1.026289	0.9910995	1.062727	1.47E-01

Note: The red font that appears in the table indicates statistical significance for the analysis results ($P < 0.05$).

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involved in the composition of the body's circadian rhythm system to feed back to form different sleep characteristics. It periodically regulates its own transcription to maintain homeostasis at the cellular level of organization.[35] Unfortunately, there is still a lack of direct observational studies to support the impact of sleep duration on hypothalamic hormone secretion. Through two-sample MR analysis, we identified a strong association between genetic variants related to long sleep, short sleep, and chronotype and hypothalamo-hypophyseal system function. Both excessively long sleep duration (greater than 9 hours) and insufficient short sleep duration (less than 6 hours) were detrimental to the secretion of hypothalamic PrRP. Vas et al's latest animal study found that PrRP plays a critical role in regulating sleep and affective states, and normal PrRP signaling appears to protect Wistar rat models from stress-induced damage. However, persistent sleep deprivation can lead to overactivation of PrRP cells and depletion of PrRP protein and receptors,[36] which is consistent with the findings of this study. Due to the anterior pituitary, PRL secretion is regulated by a variety of other modalities in addition to being stimulated by hypothalamic prolactin-releasing hormone. For example, TRH, vasopressin, and oxytocin are able to stimulate PRL release under certain conditions,[37] and considering that the hypothalamus exerts mainly inhibitory effects on PRL, the pituitary may maintain PRL at normal levels through feedback regulation by other circuits. This explains why our study did not observe any evidence of a causal relationship between sleep duration and PRL secretion at the pituitary level.

With increasing age, sleep quality tends to become progressively shallow and fragmented, and the physiological secretion of growth hormone, which is closely related to sleep, also decreases.[38] In a prospective study involving seven healthy elderly participants, Ralf-Michael Frieboes' team administered a random double-blind experimental approach, providing volunteers with hourly infusions of 50 μg of SRIF-14 medication. The results of the study revealed an interesting phenomenon: a significant reduction in total sleep time and rapid eye movement sleep after SRIF administration, suggesting that SRIF causes sleep deterioration in older adults.[39] Our study found that prolonged sleep reduces the secretion of growth hormone, which aligns with the above findings. Growth hormone, a regulatory hormone closely related to sleep, is mainly regulated by the relative actions of GH-releasing hormone and SRIF. Pulsatile growth hormone secretion has been confirmed to typically increase during the first half of nighttime sleep, coinciding with the onset of deep sleep.[40] Unfortunately, there is no whole genome sequencing data on growth hormone-releasing hormone, so the present study can only be unilaterally verified by the level of growth inhibitory hormone release.

Thirteen years ago, Christoph Randler conducted a study on morning wake-up timings and the cortisol awakening response in adolescent populations and found that individuals with evening types had significantly higher salivary cortisol levels than those with morning types.[41] Despite the relatively young age of the study participants (mean age of the adolescents was 14.02 years, SD=0.77, range 13–16, and 23.94 years in adults, SD=3.77, range 20–39), the results are still consistent with the phenomenon observed in our study. Similar results were observed by Imani et al, fractured sleep and sleep deprivation resulted in immediate activation of the organism's autonomic nerves, which subsequently reduced HPA axis activity.[42] We observed that at the level of the upstream center of the HPA axis, the early rise chronotype was detrimental to the secretion of corticotropin-releasing hormone. At the downstream target organ level, cortisol production was similarly inhibited by short sleep and early rise chronotype.

Interestingly, our study found that shorter sleep duration can effectively promote P4 production (regardless of gender), which is in direct contrast to the findings of Nolan et al's research.[43] Nolan concluded that micronized P4 can improve sleep onset latency (effect size=7.10; CI=1.30 to 12.91), increase total sleep duration, and enhance sleep quality and efficiency. To clarify whether there is a reverse causal association between the two, we performed a bidirectional MR analysis, which demonstrated that short sleep duration affects P4 production from a single direction. No potential causal association was observed after sex stratification (see Table 3 for details). We believe the reason for the different results is that Nolan's study population focused primarily on postmenopausal women with a relatively small sample size, which also had a significant single-sex bias. Coupled with the fact that their study was primarily focused on exploring the biological efficacy of P4 interventions for sleep quality, there was also significant directional specificity, which led to the opposite findings in our study. Therefore, multicenter observational studies with larger sample sizes are still needed to clarify the role of short sleep on P4 secretion.

There is compelling evidence that females appear to be more susceptible to sleep disturbances than males, and sleep disturbances have a greater physiological impact on females.[44] However, few prospective observational studies have

focused on the effects of sleep traits on gonadal steroid hormones. A prospective follow-up of 259 menstruation-stabilized women by Kara A. Michels' team indicated that women who suffered sleep deprivation had lower circulating TT levels,[45] which is also similar to our findings. However, Michels did not find any differences in average hormone concentrations related to chronotype. This may be due to the blood samples used for the study being collected during menstruation, where physiological fluctuations in estrogen and P4 levels could introduce bias in hormone measurements, making it challenging to fully represent women's normal circulating hormone levels objectively. Chronotype not only affects testosterone secretion in females but also shows a significant causal relationship with estrogen secretion. An observational study by Beata Peplonska, involving 345 premenopausal and 187 postmenopausal nurses, suggests that among premenopausal nurses, a higher frequency of night shifts per month is associated with lower circulating estrogen levels.[46] The similar conclusion that chronotype is detrimental to TT secretion has also been observed in male cohort. In a study involving male participants, Christoph Randler found a significant negative correlation between Composite Scale of Morningness (CSM) scale scores and TT levels ($r(s)=-0.220$, $p=0.023$, two-tailed test). However, there was no significant relationship between TT levels and average sleep duration,[47] which further supports our research findings. BT, as a binding product of free testosterone and albumin, can effectively reflect the biometabolic status of testosterone in vivo.[48] Similar results have also been observed in circulating BT. Our study found that circulating TT and BT levels were decreased in the early-rise chronotype population without showing significant gender specificity. In contrast, the reverse MR analysis suggested that only the female cohort was found to have circulating TT and BT levels that have a reverse effect on chronotype and sleep duration. Given the limited and controversial results from previous studies, further research with a larger sample size is necessary to validate our findings. However, it is undeniable that sleep traits exhibit significant gender specificity, and females' health status seems to be more vulnerable to the effects of changes in sleep traits. And in the future, MR analysis, as a promising new class of research modality, can be used to explore the relationship between sleep structure and psychiatric and metabolic lineage disorders.[49]

Admittedly, this study has some limitations. First, in our pursuit of data completeness, the exposure data were sourced from multiple different genome-wide studies, and the differences in study populations may introduce some data bias to our research. Second, our study primarily focused on exploring the expression levels of circulating hormones to investigate the function of the hypothalamo-hypophyseal system. However, relying solely on the secretion capacity of HPTGA may not fully represent its biological functionality. Therefore, further confirmation through animal and clinical studies is warranted. Last, all sleep trait events were self-reported rather than validated by questionnaires or objective measurements, which may lead to exposure misclassification. This aspect deserves careful attention. Future studies could attempt to use actigraphy to pinpoint measurements of sleep.[50]

Conclusion

In conclusion, our study found a clear causal association between sleep traits at the level of genetic variation and the function of the hypothalamo-hypophyseal system. The early-onset sleep chronotype was causally associated with suppression of gonadotropic function (reduced levels of TT and TB secretion) in adult humans, and this negative potency does not appear to be significantly sex specific. Extreme sleep habits are also detrimental to the maintenance of normal physiological function in the HPTGA, and the female cohort appears to be more susceptible to the effects of unfavorable sleep traits. We did not find significant evidence in our study to support any potential causal association of insomnia in maintaining the function of the hypothalamo-hypophyseal system. Maintaining healthy and regular sleep habits is essential for HPTGA to maintain normal biological functions. The present study also provides new insights into the biological regulatory mechanisms of HPTGA as well as related clinical research, and further large-sample investigations are urgently needed to clarify the potential association between sleep traits and HPTGA.

Abbreviations

MR, Mendelian randomization; CRFBP, corticotropin-releasing factor-binding protein; PRL, prolactin; DHEA, dehydroepiandrosterone; TT, total testosterone; BT, bioavailable testosterone; FT4, free thyroxine; P4, progesterone; PrRP, prolactin-releasing peptide; SRIF, somatostatin; E2, estradiol; HPTGA, hypothalamic-pituitary-target gland axis; SNPs,

single-nucleotide polymorphisms; TRH, thyrotropin-releasing hormone; GnRH, gonadotropin-releasing hormone; GH, growth hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; TSH, thyroid stimulating hormone; ACTH, adrenocorticotropic hormone; TPO, thyroid peroxidase; Tg, thyroglobulin; NHSBT, England's National Health Service Blood and Transplant; SHBG, sex hormone-binding globulin; ALD, IVs, aldosterone; instrumental variables; MAF, minor allele frequency; LD, linkage disequilibrium; ML, maximum likelihood; IVW, inverse variance weighted; HPO, hypothalamic-pituitary-ovarian; HPT, hypothalamic-pituitary-thyroid; HPA, hypothalamic–pituitary–adrenal.

Data Sharing Statement

The datasets analysed in this study are publicly available summary statistics. Genetic instruments can be obtained from the individual referenced papers.[19–21] All summary data of HPTGA can be downloaded from the following website: UK Biobank database (https://www.nealelab.is/uk-biobank), the IEU Open GWAS project (https://gwas.mrcieu.ac.uk/), the Thyroidomics Consortium (https://transfer.sysepi.medizin.uni-greifswald.de/thyroidomics/) and the Zenodo database (https://zenodo.org/).

Ethics Approval and Consent to Participate

All the contents of this study have been reviewed and approved by the Ethics Committee of the second affiliated hospital of Chongqing medical university and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Disclosure

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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