

# Chronotype and cognitive function: Observational study and bidirectional Mendelian randomization



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## Summary

**Background** Association has been found between chronotype and cognitive function in conventional observational studies but whether this association is causal and if so, its direction, is uncertain. There are also concerns among people with later chronotype that their habits may be detrimental to cognitive function.

**Methods** We analyzed the association between chronotype (measured as sleep midpoint) and cognitive function (measured by Mini-Mental Status Examination (MMSE) and Delayed Word Recall Test (DWRT)) using multivariable linear regression on 14,582 participants in the Guangzhou biobank cohort study (GBCS) from 2008 to 2012. Using bidirectional Mendelian randomization, we used 207 single nucleotide polymorphisms (SNPs) associated with chronotype from the combination of UK Biobank and 23andMe (n = 697,828), and 127 SNPs associated with cognitive function from the combination of UK Biobank and COGENT consortium (n = 257,841).

**Findings** Observationally in GBCS, later chronotype was associated with better cognitive function (MMSE scores:  $\beta = 0.14$  per hour; 95% confidence interval (CI), 0.09–0.19; DWRT scores:  $\beta = 0.07$  per hour; 95% CI, 0.04–0.11). Bidirectional MR showed genetic predisposition to early, versus later, chronotype was not associated with cognitive function using inverse-variance weighted ( $\beta = -0.02$ ; 95% CI, -0.05 to 0.01). However, better cognitive function was associated with decreased odds of early chronotype (UK Biobank: odds ratio = 0.88 per standardized score; 95% CI, 0.83–0.93; 23andMe: 0.87 per standardized score; 95% CI, 0.80–0.95).

**Interpretation** It is a reassuring finding for adults with later chronotype who may be concerned if such a habit has a negative impact on cognitive function.

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**Keywords:** Chronotype; Cognitive function; Causality; Older people

## Introduction

Chronotype is characterized as a behavioral manifestation of our internal timing system in 24-h day–night cycle, the circadian rhythm.<sup>1</sup> Chronotypes are typically classified into evening-type and morning-type, otherwise known as “night owls” and “early larks”.<sup>2</sup> Chronotype has been found in conventional observational studies to

be associated with various diseases including metabolic<sup>3</sup> and psychiatric disorders,<sup>4</sup> as well as brain physiology<sup>5</sup> and cognitive function.<sup>6</sup> People with later chronotype may be concerned if such a habit has a negative impact on cognitive function.<sup>7,8</sup>

Reported associations in previous studies (Table S1) varied by age group. However, conventional

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### Research in context

#### Evidence before this study

We searched PubMed and Web of Science in title and abstract using keywords (“cognitive” or “cognition”) AND (“chronotype” or “morningness” or “eveningness” or “sleep midpoint” or “circadian rhythms”) up to August 2022 and identified twenty studies on the associations of chronotype and cognitive function. Generally, evening-type was associated with better cognitive performance across a wide age range, although inconsistent results were reported by some studies using varying definitions of cognitive function. As conventional observational studies cannot completely rule out reverse causality and residual confounding, the causality and direction of the association between chronotype and cognitive function is still uncertain.

#### Added value of this study

In the large cohort of older Chinese, later chronotype was associated with better cognitive function using conventional regression analyses. In bidirectional two-sample Mendelian randomization (MR) studies, better cognitive function led to later chronotype in adults, but no evidence for any impact of chronotype on cognitive function was found. To our knowledge, this is the first study that provides robust evidence for this causal relationship and its direction of the association between chronotype and cognitive function.

#### Implications of all the available evidence

Better cognitive function led to later chronotype, but not vice versa. It is a reassuring finding for adults with later chronotype who may be concerned if such a habit has a negative impact on cognitive function.

observational study cannot completely rule out reverse causality and residual confounding, which makes it hard to infer causality. In addition, randomized control trials (RCTs) might have ethical issues. Given chronotype was found to be heritable in twin and family studies,<sup>9</sup> Mendelian randomization (MR) analysis using genetic variants as instrumental variables represents a potentially robust way to examine the causal nature of the association between chronotype and cognitive function.<sup>10</sup> As genetic variants are randomly allocated at conception, MR is less affected by confounding and can produce results analogous to RCTs. A recent study using MR with limited genetic variables showed no effect of chronotype on the risk of Alzheimer’s disease (AD).<sup>11</sup> However, this study did not report results on cognitive function.<sup>11</sup>

In young and middle-aged adults, circadian preference is often influenced by adherence to school/work schedules, which may generate confounding. Older people, especially those who have retired, have chronotype closer to the natural sleep timing preference. Investigations on the association between chronotype and cognitive function in older people would provide more reliable evidence for causal inference. Therefore, in this study, we analyzed the associations between chronotype and cognitive function using conventional regression and bidirectional two-sample MR to analyze the association and clarify the direction for causal inference between chronotype and cognitive function.

## Methods

### Conventional observational study

#### Study participants

The Guangzhou biobank cohort study (GBCS) is a 3-way collaboration among Guangzhou Twelfth People’s Hospital and the Universities of Hong Kong, China and

Birmingham, UK. 30,430 participants were recruited from 2003 to 2008. Of them, 18,129 participants returned for follow-up examination from 2008 to 2012 with a median age of 65 years (range 53–98). Almost all participants were retired (rate 99.6%).<sup>12</sup> In the current study, the chronotype information was collected in the follow-up survey. Details of GBCS have been reported elsewhere.<sup>13</sup> Briefly, recruitment of GBCS participants was from “The Guangzhou Health and Happiness Association for the Respectable Elders” (GHHARE), a community social and welfare organization. Membership is open to local residents aged 50+ years for a minimal nominal fee (about 50 US cents) per month. The GHHARE included about 7% of residents in this age group across all 10 districts of Guangzhou. The baseline and follow-up examination included a computer-based face-to-face interview by nurses. Information of demographic characteristics, lifestyle factors, family and personal medical history, and detailed assessment of anthropometric parameters, blood pressure, fasting plasma glucose, lipids and inflammatory markers was collected.

#### Chronotype

The Pittsburgh Sleep Quality Index (PSQI) questionnaire was used to assess the sleep information, in which the sleep bedtime was assessed by the following question: “During the past month, when have you usually gone to bed at night?” and wake-up time by the question “During the past month, when have you usually gotten up in the morning?”. Sleep midpoint was used to assess the chronotype in GBCS. Sleep midpoint (hours: minutes) was defined as the midpoint between the start and end of a given sleep period, and was treated as a continuous variable on the per-hour scale (e.g. 2:30 = 2.5 h) in data analysis. Sleep midpoint considered both sleep duration and timing, with later midpoints

indicating higher probability of being an evening person.<sup>14,15</sup> Then, participants were classified into three groups (morning-type, intermediate and evening-type) by tertiles of sleep midpoint: morning-type (before 2:00 AM), intermediate (2:00 AM–2:30 AM) and evening-type (after 2:30 AM).

#### *Cognitive function*

Cognitive function was assessed by Mini-Mental State Examination (MMSE)<sup>16</sup> and Delayed Word Recall Test (DWRT). MMSE is a 30-point test that has been used extensively to measure cognitive functions including registration (repeating named prompts), attention and calculation, recall, language, ability to follow simple commands and orientation, with scores ranging from 0 to 30. Higher scores indicate better cognitive function. The DWRT is a test of verbal learning and recent memory requiring recall of a word list after a 5 min delay. First, 10 simple Chinese words (soy sauce, arm, letter, chairman, ticket, grass, corner, stone, book and stick) were read out to the participants one-by-one, pausing for 1 s between each. Participants recalled the words they heard immediately after the last word. This procedure was done for three times, and a 5-min delay was given after the third time. The DWRT score was the number of correct words of the 5-min delayed recall ranging from 0 to 10, with higher scores indicating better cognitive function.

#### **Mendelian randomization**

##### *Genetic associations with chronotype*

We obtained single nucleotide polymorphisms (SNPs) strongly associated with chronotype from the largest and most recent genome-wide association study (GWAS) of a combined study including 23andMe and UK Biobank, with 697,828 European-ancestry participants.<sup>17</sup> The GWAS from UK Biobank included 449,734 participants with adjustments of sex, age, study center and a derived variable representing genotyping release. The GWAS performed by 23andMe included 248,098 participants. The definition of chronotype in UK Biobank and 23andMe is in [Table S2](#).

##### *Genetic associations with cognitive function*

The genetic instruments of chronotype were applied to the largest publicly available GWAS of cognitive function from COGENT Consortium (35 sub-cohorts) and UK Biobank, with 257,841 European-ancestry participants, adjusted for sex, age and population stratification.<sup>18</sup> The definition of cognitive function in COGENT Consortium and UK Biobank is in [Table S2](#).

#### **Ethics statement**

The Guangzhou Medical Ethics Committee of the Chinese Medical Association approved the study. Informed

consent was obtained from all individual participants included in the study. The Mendelian randomization study is an analysis of publicly available summary data that does not require ethical approval. This research was done without public involvement. Participants were not invited to comment on the study design and were not consulted to develop relevant outcomes or interpret the results. Participants were not invited to contribute to the writing or editing of this paper for readability or accuracy.

#### **Statistical analysis**

##### *Conventional observational study*

We used Kruskal–Wallis test to compare levels of sleep midpoint, MMSE and DWRT scores by GBCS participants' characteristics, since these three variables were non-normally distributed. Multivariable linear regression was used to analyze the adjusted associations of sleep midpoint (in hours) with cognitive function, giving adjusted regression coefficients and 95% confidence interval (CI). No factor was adjusted in crude model. Potential confounders adjusted in the model 1 were sex and age. Model 2 was additionally adjusted for education ( $\leq$ primary, middle school,  $\geq$ college), occupation (manual, non-manual, other), body mass index (BMI), smoking status (never, former, current), alcohol use (never, former, current), physical activity (inactive, minimally active, active) and self-reported health (good, average, poor). Model 3 was additionally adjusted for self-reported history of cardiovascular disease and diabetes. Model 4 was additionally adjusted for depressive symptoms [15-item Geriatric Depression Scale (GDS-15)] and sleep duration. Since the proportion of missing data in all variables was very low (i.e., less than 8.7%) ([Table S3](#)), the complete case analysis was used in the current study. We also replicated the results using multiple imputation as sensitivity analysis. Besides, we assessed if the associations of sleep midpoint with MMSE and DWRT scores varied by sex, age, education, occupation, BMI, self-reported health, alcohol use, smoking status, physical activity, cardiovascular disease history, diabetes history and depressive symptoms. Moreover, we also assessed if the associations of sleep midpoint with MMSE and DWRT scores were independent of the APOE  $\epsilon$ 4 allele, the established genetic risk factor for cognitive decline.

##### *Mendelian randomization*

The bidirectional causal associations between sleep midpoint and cognitive function were assessed using bidirectional Mendelian randomization. We estimated the F-statistic for each SNP by the square of the effect of SNP on exposure divided by the variance of SNP on exposure, using the approximation described by Bowden and colleagues,<sup>19</sup> and generated the mean F-statistic for exposure. Linkage disequilibrium between SNPs was

identified using the “ld\_clump” R package. To check for unknown pleiotropic effects statistically we used MR-Egger and the Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) test.<sup>20</sup> Wald estimate for independent SNPs ( $r^2 < 0.001$ ) was calculated as the estimate for a SNP on outcome divided by the estimate for the SNP on exposure.<sup>21</sup> We combined Wald estimates using inverse-variance weighted (IVW) with random effects. Cochran’s Q test was used to assess heterogeneity.<sup>22</sup>

All statistical analysis was conducted in Stata version 16.0 (StataCorp LP, College Station, TX), and R version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria) using the “TwoSampleMR”, “MendelianRandomization” and “MRPRESSO” packages. All tests were two-sided, with  $P < 0.05$  as statistically significant.

#### Role of funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors (LX and KKC) had full access to all of the data in the study, compiled the database for analysis, and had final responsibility for the decision to submit for publication.

## Results

### Conventional observational study

As data of sleep midpoint was not available at baseline, we used data from 18,104 participants who returned for follow-up examination from 2008 to 2012 in this study. After excluding participants with missing data, 14,587 (80%) participants were included. [Table 1](#) shows that participants with younger age, higher education, non-manual occupation, more active physical activity and more depressive symptoms had later sleep midpoint and higher MMSE scores (all  $P < 0.007$ ). Participants with higher education and more depressive symptoms had higher DWRT scores ( $P < 0.001$ ). Women had later sleep midpoint than man ( $P = 0.014$ ). Participants with diabetes history had earlier sleep midpoint and lower MMSE scores ( $P < 0.001$ ).

[Table 2](#) shows that in unadjusted model, participants with per hour later sleep midpoint had higher MMSE ( $\beta = 0.51$ ; 95% CI, 0.46–0.56) and DWRT scores (0.28; 0.24–0.31). The association attenuated but remained significant after adjusting for sex, age, education, occupation, BMI, self-reported health, alcohol use, smoking status, physical activity, cardiovascular disease history, diabetes history, depressive symptoms and sleep duration (MMSE scores: 0.14; 95% CI, 0.09–0.19; DWRT scores: 0.07; 95% CI, 0.04–0.11) (model 4). Similar results were found using multiple imputation in sensitivity analysis ([Table 2](#) and [Table S4](#)). Similar dose-response positive associations were found when treating sleep midpoint as categorical variable (morning-

type, intermediate and evening-type). No interaction between sleep midpoint and APOE  $\epsilon 4$  was found ([Table S5](#)). We found significant interactions by sex, age, education, occupation, self-reported health, alcohol use, smoking status, physical activity, cardiovascular disease history and depressive symptoms in the association between sleep midpoint and MMSE score (all  $P < 0.008$ ) ([Fig. S1](#)), and significant interactions by age, education, alcohol use and cardiovascular disease history in the association between sleep midpoint and DWRT score (all  $P < 0.002$ ) ([Fig. S2](#)).

### Bidirectional Mendelian randomization

A total of 351 SNPs associated with chronotype (morning person) at genome-wide significance ( $P < 5 \times 10^{-8}$ ) were obtained. Of 351 SNPs, 135 SNPs were dropped due to absence from linkage disequilibrium reference panel or high linkage disequilibrium ( $r^2 > 0.001$ ). The remaining 216 SNPs were available in outcome dataset COGENT consortium and UK Biobank. Of them, 9 palindromic SNPs were dropped, giving 207 independent SNPs in the main analysis. The average F-statistic of the genetic instruments for chronotype was 59.26. A total of 225 SNPs associated with cognitive function ( $P < 5 \times 10^{-8}$ ) were obtained from the most recent published GWAS (7–10% of the variance). Of 225 SNPs, 93 SNPs were dropped due to absence from linkage disequilibrium reference panel or high linkage disequilibrium ( $r^2 > 0.001$ ), 5 SNPs were dropped because they were palindromic in both UK Biobank and 23andMe, giving 127 SNPs used in the final analysis. The F-statistic of these genetic instruments for cognitive function was 44.01. [Fig. S3](#) shows the selection of these SNPs related to chronotype and cognitive function. [Tables S6 and S7](#) show summary information on the genetic instruments for chronotype and cognitive function used in two-sample Mendelian randomization analysis.

[Table 3](#) shows that genetically predicted being a morning person was not associated with cognitive function ( $\beta = -0.02$ ; 95% CI,  $-0.05$  to  $0.01$ ,  $P = 0.15$ ) using IVW with random-effects. Sensitivity analyses using MR-Egger, WM, and the corrected MR-PRESSO showed consistent null results. No evidence for directional pleiotropy was found ( $P$  for MR-Egger intercept = 0.25). However, in the opposite direction, genetically determined better cognitive function was significantly associated with the decreased odds of being a morning person using IVW (UK Biobank: OR = 0.88; 95% CI, 0.83–0.93,  $P < 0.001$ ; 23andMe: OR = 0.87; 95% CI, 0.80–0.95,  $P < 0.001$ ). Similar results were found using WM and the corrected MR-PRESSO. No evidence of directional pleiotropy was found ( $P$  for the MR-Egger intercept = 0.17 in UK Biobank and = 0.21 in 23andMe). The results were similar after excluding potential pleiotropic SNPs (11 SNPs were removed when

Characteristic	Number (%)	Sleep midpoint		MMSE scores		DWRT scores	
		Median (IQR)	P	Median (IQR)	P	Median (IQR)	P
<b>Sex</b>							
Men	3100 (21.3)	2:15 (1:03)	0.014	29 (3)	0.036	6 (2)	<0.001
Women	11,487 (78.7)	2:18 (1:12)		29 (3)		6 (3)	
<b>Education</b>							
≤Primary	5621 (38.5)	2:00 (1:00)	<0.001	28 (4)	<0.001	6 (3)	<0.001
Middle school	7641 (52.4)	2:30 (1:00)		29 (2)		7 (3)	
≥College	1325 (9.1)	2:30 (1:00)		29 (2)		7 (3)	
<b>Occupation</b>							
Manual	8763 (60.1)	2:09 (1:06)	<0.001	28 (4)	<0.001	6 (2)	<0.001
Non-manual	3442 (23.6)	2:30 (1:00)		29 (3)		6 (3)	
Other	2382 (16.3)	2:30 (1:00)		29 (3)		6 (3)	
<b>Alcohol use</b>							
Never	6846 (46.9)	2:09 (1:09)	<0.001	28 (3)	<0.001	6 (2)	<0.001
Former	35 (0.3)	2:05 (1:30)		27 (3)		6 (3)	
Current	7706 (52.8)	2:30 (1:00)		29 (3)		6 (3)	
<b>Smoking status</b>							
Never	12,597 (86.4)	2:18 (1:12)	<0.001	29 (3)	0.008	6 (2)	<0.001
Former	1730 (11.9)	2:09 (1:00)		28 (3)		6 (2)	
Current	260 (1.7)	2:30 (1:18)		28 (2)		6 (2)	
<b>Physical activity</b>							
Inactive	245 (1.7)	2:09 (1:00)	0.007	27 (3)	<0.001	6 (2)	0.009
Minimally active	2553 (17.5)	2:15 (1:06)		28 (3)		6 (2)	
Active	11,789 (80.8)	2:18 (1:12)		29 (3)		6 (2)	
<b>Self-reported health</b>							
Good	2351 (16.1)	2:15 (1:00)	0.002	28 (2)	<0.001	6 (3)	<0.001
Average	10,719 (73.5)	2:18 (1:12)		29 (3)		6 (2)	
Poor	1517 (10.4)	2:09 (1:06)		28 (4)		6 (3)	
<b>Diabetes history</b>							
No	12,959 (88.8)	2:18 (1:12)	<0.001	29 (3)	<0.001	6 (2)	0.002
Yes	1628 (11.2)	2:09 (1:00)		28 (4)		6 (2)	
<b>Cardiovascular disease history</b>							
No	7011 (48.1)	2:18 (1:12)	0.086	29 (3)	0.004	6 (2)	<0.001
Yes	7576 (51.9)	2:18 (1:00)		28 (3)		6 (2)	
		<b>β<sup>a</sup> (95% CI)</b>	<b>P</b>	<b>β<sup>b</sup> (95% CI)</b>	<b>P</b>	<b>β<sup>c</sup> (95% CI)</b>	<b>P</b>
Age, years	14,587	-0.04 (-0.04, -0.03)	<0.001	-0.12 (-0.12, -0.11)	<0.001	-0.07 (-0.07, -0.06)	<0.001
Body mass index, kg/m <sup>2</sup>	14,587	0.00 (-0.01, 0.00)	0.41	-0.02 (-0.04, -0.01)	0.002	-0.01 (-0.02, 0.00)	0.13
Depressive symptoms (GDS-15)	14,587	0.04 (0.03, 0.05)	<0.001	0.25 (0.22, 0.28)	<0.001	0.14 (0.12, 0.17)	<0.001
Sleep duration, hours	14,587	0.00 (0.00, 0.00)	0.931	0.00 (-0.01, 0.00)	0.274	0.00 (0.00, 0.00)	0.261

IQR: interquartile range; CI: confidence interval; GDS-15: 15-item Geriatric Depression Scale. <sup>a</sup>Dependent variable: sleep midpoint; hours. <sup>b</sup>Dependent variable: MMSE scores; higher scores indicating better cognitive function. <sup>c</sup>Dependent variable: DWRT scores; higher scores indicating better cognitive function.

**Table 1: Association of sleep midpoint, mini-mental state examination scores (MMSE scores) and delayed word recall test scores (DWRT scores) with demographic characteristics, lifestyle factors and disease history in 14,587 participants of the Guangzhou biobank cohort study.**

chronotype was exposure, 8 SNPs were removed when cognitive function was exposure) (Table S8). Fig. 1 shows the overview of the design and main results of this study.

### Discussion

In the cohort of older Chinese, later chronotype was associated with better cognitive function in conventional

regression analyses. In bidirectional Mendelian randomization analyses, we show that better cognitive function leads to later chronotype, but not vice versa. To our knowledge, this is the first study that provides robust evidence for this causal relationship and its direction.

Previous studies on the associations between chronotype and cognitive function across different age groups reported inconsistent results (Table S1). A

Outcomes	Exposures	Crude model		Model 1		Model 2		Model 3		Model 4	
		β (95% CI)	P	Adjusted β (95% CI)	P	Adjusted β (95% CI)	P	Adjusted β (95% CI)	P	Adjusted β (95% CI)	P
MMSE scores	Sleep midpoint	0.51 (0.46, 0.56)	<0.001	0.25 (0.20, 0.29)	<0.001	0.15 (0.10, 0.19)	<0.001	0.14 (0.10, 0.19)	<0.001	0.14 (0.09, 0.19)	<0.001
DWRT scores		0.28 (0.24, 0.31)	<0.001	0.15 (0.11, 0.18)	<0.001	0.08 (0.04, 0.11)	<0.001	0.08 (0.04, 0.11)	<0.001	0.07 (0.04, 0.11)	<0.001
MMSE scores	Morning-type	0 (reference)		0 (reference)		0 (reference)		0 (reference)		0 (reference)	
	Intermediate	0.69 (0.59, 0.80)	<0.001	0.35 (0.25, 0.45)	<0.001	0.19 (0.06, 0.32)	0.003	0.19 (0.06, 0.32)	0.003	0.19 (0.08, 0.33)	0.001
	Evening-type	1.14 (1.04, 1.24)	<0.001	0.50 (0.40, 0.59)	<0.001	0.18 (0.05, 0.30)	0.004	0.18 (0.06, 0.30)	0.004	0.20 (0.08, 0.32)	0.001
DWRT scores	Morning-type	0 (reference)		0 (reference)		0 (reference)		0 (reference)		0 (reference)	
	Intermediate	0.41 (0.33, 0.48)	<0.001	0.25 (0.17, 0.33)	<0.001	0.12 (0.01, 0.22)	0.03	0.12 (0.01, 0.22)	0.03	0.12 (0.02, 0.23)	0.02
	Evening-type	0.62 (0.55, 0.69)	<0.001	0.35 (0.27, 0.42)	<0.001	0.13 (0.02, 0.23)	0.02	0.13 (0.02, 0.23)	0.02	0.14 (0.04, 0.24)	0.008

Crude model: no adjustment. Model 1: additionally adjusting for sex, age. Model 2: additionally adjusting for sex, age, education, occupation, self-reported health, BMI, smoking status, alcohol use, physical activity. Model 3: additionally adjusting for cardiovascular disease history and diabetes history. Model 4: additionally adjusting for depressive symptoms, sleep duration. CI: confidence interval.

**Table 2: Association of sleep midpoint with mini-mental state examination scores (MMSE scores) and delayed word recall test scores (DWRT scores) in Guangzhou biobank cohort study.**

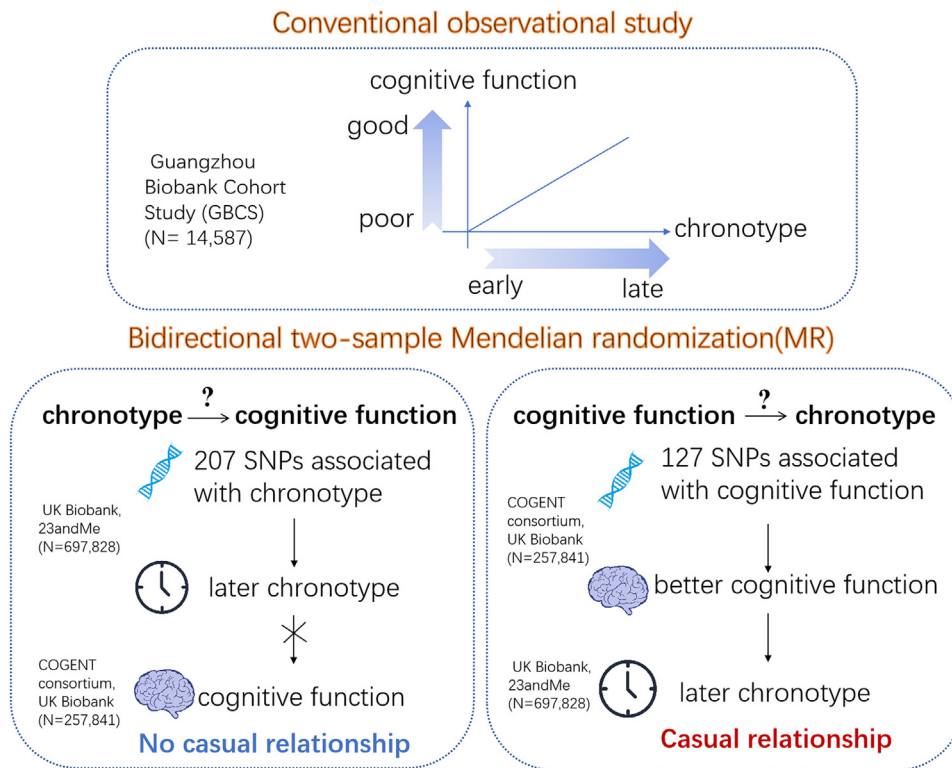
meta-analysis of 26 studies including participants with an average age from 9 to 43 years found no significant association between morningness and cognitive function in the overall sample.<sup>23</sup> However, subgroup analysis by age showed that eveningness was associated with better cognitive function in adults aged 25+ years. This study did not report result on older adults (e.g. 65+ years old). A cross-sectional study in UK Biobank of 477,529 individuals aged 40–69 years found that compared with morning chronotype, evening chronotype was associated with better cognitive function.<sup>24</sup> Another longitudinal study of 2893 Korean aged 60+ years found that compared with those with intermediate chronotype at both baseline and follow-up, participants with later chronotype at baseline and follow-up had lower risk of cognitive decline during the 4-year follow-up period.<sup>25</sup> Our MR results were consistent with findings of these

two studies above<sup>24,25</sup> and added by providing causal effects of cumulative exposures across the life course, rather than the effects at a specific short time, suggesting that lifetime better cognitive function is associated with later chronotype but not vice versa. Because of the early start of school day and more sleep demand,<sup>26</sup> young students with later chronotype might suffer from “sleep deprivation”,<sup>27</sup> and thus the poor academic performance could be attributable to insufficient sleep duration or poor sleep quality.<sup>28,29</sup> Short sleep duration was linked to a higher risk of dementia,<sup>30</sup> but we found similar results after adjusting for sleep duration, indicating the association between chronotype and cognitive function in older people was independent of sleep duration. A recent MR study showed that genetic predisposition to AD was significantly associated with being a “morning person”,<sup>11</sup> similar to what we found with

Exposures	Outcomes	SNP	F statistic	Methods	β (95% CI)	P	IVW	MR-Egger Intercept (P)
							Cochran's Q statistic (I <sup>2</sup> )	
Chronotype: morning person (reference: evening person) (UK Biobank, 23andMe)	Cognitive function (standardized score of cognitive tests) (COGENT consortium, UK Biobank)	207	59.26	IVW	-0.02 (-0.05, 0.01)	0.15	937.3 (78.0%)	-0.001 (0.25)
				WM	-0.01 (-0.04, 0.01)	0.27		
				MR-Egger	0.02 (-0.06, 0.10)	0.61		
				MRPRESSO	-0.01 (-0.04, 0.01)	0.26		
<b>OR (95% CI)</b>								
Cognitive function (standardized score of cognitive tests) (COGENT consortium, UK Biobank)	Chronotype: morning person (reference: evening person) (UK Biobank)	127	44.01	IVW	0.88 (0.83, 0.93)	<0.001	636.7 (80.2%)	-0.004 (0.17)
				WM	0.85 (0.81, 0.90)	<0.001		
				MR-Egger	1.03 (0.82, 1.32)	0.76		
				MRPRESSO	0.87 (0.83, 0.91)	<0.001		
Cognitive function (standardized score of cognitive tests) (COGENT consortium, UK Biobank)	Chronotype: morning person (reference: evening person) (23andMe)	127	44.01	IVW	0.87 (0.80, 0.95)	0.002	567.7 (77.2%)	-0.005 (0.21)
				WM	0.84 (0.77, 0.90)	<0.001		
				MR-Egger	1.10 (0.75, 1.60)	0.62		
				MRPRESSO	0.87 (0.80, 0.93)	<0.001		

OR: odd ratio; CI: confidence interval; IVW: inverse-variance weighted; WM: weighted median method; MR-PRESSO: Mendelian randomization pleiotropy residual sum and outlier.

**Table 3: Association between chronotype and cognitive function using bidirectional Mendelian randomization.**



**Fig. 1:** Overview of the design and main results of this study.

cognitive function (a preclinical predictor for AD) as the variable of interest. The similar association of AD and preclinical stage of AD (i.e., poor cognitive function) with chronotype provides support for a causal relationship.

If the association between cognitive function and chronotype is casual, there are several potential mechanisms. Previous studies have revealed the molecular and cellular link between chronotype and respective physiological processes in humans.<sup>31,32</sup> People's cognitive performance (working memory and attention) along with their electrophysiological components are significantly enhanced at the circadian-preferred time. Therefore, people may establish a specific chronotype depending on their "circadian-preferred time" when having enhanced cortical excitability and better learning and cognitive functions.<sup>33</sup> According to the theoretical hypothesis in evolutionary psychology, more intelligent individuals are more likely to be nocturnal than less intelligent individuals.<sup>34</sup> However, the mechanisms for the association of cognitive function with chronotype are still unclear. Future studies about the origin of preference in circadian rhythms are clearly necessary.

One strength of our study is that we used two study designs which showed consistent results. Also, by integrating data from >200,000 individuals, our MR study was well powered to detect small effects. Interpretation

of the results from the MR analyses requires considerations for three assumptions (i.e., relevance, independence and exclusion restriction). Relevance requires that the genetic instruments strongly predict the exposure ideally on functional as well as statistical grounds. In our study, the SNPs for chronotype and cognitive function all reached genome-wide significance with high average F-statistic (59.26 for chronotype and 44.01 for cognitive function). Moreover, genomic control and participants exclusively of European descent reduce the likelihood of confounding by population stratification, supporting independence. To assess pleiotropy bias, we found no statistical evidence from MR-Egger testing. Despite the  $I^2$  statistic suggesting a high level of heterogeneity, the weighted median and IVW provided consistent estimates. MR-PRESSO indicated some outliers, but the main findings were similar before and after excluding them. Another limitation is that there was partial sample overlap among GWAS datasets on chronotype and cognitive function, which may lead to increased type 1 error and biased effect estimates towards observational estimates.<sup>35</sup> However, with relatively large sample sizes, the bias due to sample overlap is expected to be very small.<sup>36</sup> Moreover, similar estimates of the effect of cognitive function on chronotype using GWAS of chronotype from UK Biobank (32% overlap) and 23andMe (non-overlap) were found,

suggesting our results were unlikely to be due to sample overlap. Besides, in GBCS, the usual bedtime and wake-up time were assessed without separating by workdays and non-workdays. However, as almost all participants were retired at the time when assessed PSQI, the difference in sleep habits between workday and non-workday was minimal. Also, in a long-term cohort study missing data is almost inevitable. However, the proportion of missing data in the GBCS was low and we found similar results after accounting for missing data, suggesting the lost to follow-up was not a major concern. Furthermore, chronotype in our traditional regression analyses was defined by sleep midpoint time, but in MR study was defined by Morningness-Eveningness Questionnaire (MEQ). However, a previous study has shown that sleep midpoint (rather than start and end time of sleep) had high correlation ( $r = 0.7$ ) with MEQ score.<sup>37</sup> Finally, the participants were recruited from an organization whose membership was open to anyone for a low fee, affordable by most Guangzhou residents. Moreover, within sex and age group, the participants had fairly similar prevalence of chronic diseases such as diabetes and hypertension to nationally representative samples of urban Chinese,<sup>13</sup> hence the generalizability of the findings of this study to the wider population might not be a concern.

We provided, to our knowledge, the first evidence based on bidirectional MR analyses that better cognitive function leads to later chronotype, but not vice versa. We found no evidence that later chronotype led to lower cognitive function, a reassuring finding for adults with such a habit.

#### Contributors

JW, YRL, CQJ, WSZ, FZ, TZ, YLJ, THL, KKC and LX have substantial contributions to conception and design, acquisition of funding, data and interpretation of data; JW and YRL analyzed the data and drafted the article, KKC, THL and LX revised it critically for important intellectual content. KKC and LX are the guarantors. All authors contributed to final approval of the paper.

#### Data sharing statement

(1). Observational study: Ethical approval in place allows us to share data on requests. Please directly send such requests to the Guangzhou Biobank Cohort Study Data Access Committee ([gbcdata@hku.hk](mailto:gbcdata@hku.hk)). (2). Mendelian randomization study: The full set of UK Biobank-only chronotype and morning person GWAS summary statistics can be found at <https://www.ebi.ac.uk/gwas/publications/30696823>. The summary statistics of cognitive function can be found at <https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST006572/>.

#### Declaration of interests

The authors declare no conflict of interest.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.eclinm.2022.101713>.

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