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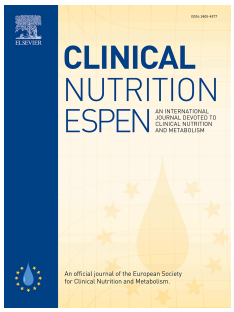

How far do we have to go on the road to calcium supplementation?

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Title：How far do we have to go on the road to calcium supplementation?

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Title：How far do we have to go on the road to calcium supplementation?

Abstract:

Background & Aim: Sleep disorder is a growing concern, and calcium supplementation is often recommended as a potential intervention for sleep disorders. However, the causal relationship between calcium levels and the incidence of sleep disorders remains unclear. Mendelian randomization techniques utilizing genetic variants that affect calcium levels, can provide valuable insights into causality. This study aims to examine the association between calcium levels and sleep disorders in a diverse population that includes both adolescents and adults, and investigate the effects of calcium levels on sleep disorders.

Methods: Mendelian randomization analysis was conducted using data from UK Biobank and FinnGen datasets. The inverse-variance weighting (IVW) was selected as the primary method. In addition, traditional mediation analysis was performed on a subset of the NHANES data spanning from 2007 to 2018.

Results: Our findings provide evidence supporting a causal relationship between calcium intake and reduced risk of sleep disorders (beta = -0.079, SE = 0.0395, P = 0.0457). While not reaching statistical significance, other MR methods such as weighted median and Mr-Egger exhibited similar directional trends. Analysis of the NHANES cohort revealed a negative association between calcium levels and the prevalence of sleep disorders in male, black, and physically active populations. However, this association was not observed in other demographic groups.

Conclusion: Our results suggested that there is no significant correlation between calcium levels and sleep disorder in non-exercise populations. This raises concerns about the long-term high-dose calcium supplementation in clinical practice, which requires further investigation.

Keywords: Sleep disorders; Calcium; Mendelian randomization

1. Introduction

Sleep quality plays a crucial role in various aspects of human functioning, including, attention, cognition, learning, memory, decision-making skills, and daytime activities [1–3]. Conversely, poor sleep quality has been associated with chronic diseases such as hypertension, hyperlipidemia, cardiovascular diseases, diabetes mellitus, obesity, and metabolic syndrome [4]. Sleep disorders including insomnia, sleep apnea, narcolepsy, and restless leg syndrome can disrupt sleep patterns and contribute to adverse health outcomes such as obesity, hypertension, type 2 diabetes, cardiovascular disease, Alzheimer's disease and increased mortality [4–6].

In recent decades, there has been a significant increase in research exploring the risk factors associated with sleep disorders and their implications for human health and well-being [7]. Multiple factors, such as inadequate intake of specific nutrients, acute and chronic diseases, and mental illness, have been associated with sleep disorders [7–10]. Previous researches have suggested a possible inverse association between calcium level and the incidence of sleep disorder [10,11]. However, these studies have relied on observational designs, limiting their ability to establish causal relationship between calcium level and sleep disorder. As a result, the causal association between calcium level and sleep disorders remains uncertain.

Mendelian randomization represents a powerful tool in epidemiological research that employs genetic variation as an instrumental variable to assess causal relationships between risk factors and specific diseases [12]. Unlike traditional observational studies, Mendelian randomization capitalizes on the random assignment of alleles to offspring, mimicking the randomization process in controlled trials [13]. This approach effectively address confounding factors and reverse causality commonly observed in observational studies, producing reliable and representative results comparable to randomized controlled trials. No previous studies have employed this approach to investigate the association between calcium levels and sleep disorders using Mendelian randomization, despite these advantages of Mendelian randomization.

Therefore, the objective of this study was to utilize a two-sample Mendelian randomization method, in conjunction with data from the NHANES cohort, to examine the potential causal relationship between calcium levels and sleep disorders. By using this

novel approach, this study aimed to establish a theoretical foundation for understanding the impact of calcium on the incidence and progression of sleep disorders. Our study was able to shed light on the relationship between calcium levels and sleep disorders, which may help guide the use of calcium supplements clinically in people with sleep disorders.

2. Methods

2.1. Study design

We conducted a two-sample Mendelian randomization analysis using a combined genome-wide association study (GWAS) dataset to evaluate the causal relationship between calcium levels and sleep disorders. To ensure the robustness of our findings, we conducted a sensitivity analysis. Additionally, we employed conventional observational mediation analysis techniques to examine the mediation effects within the NHANES 2007-2018 dataset (Figure S1).

2.2. Data sources and selection of genetic variants

For this study, we utilized data from the UK Biobank (https://gwas.mrcieu.ac.uk), which consisted of 315,153 cases accessed through the MR-base platform. Furthermore, data from the FinnGen study of sleep disorders was obtained, which included 19,155 patients and 197,545 controls, as detailed in Table 1. To identify single nucleotide polymorphisms (SNPs) that demonstrated a significant association with calcium, we applied a genome-wide threshold of $p < 5e\text{-}8$, $r2 < 0.0001$, and clump distance > 10,000 KB.

2.3. Mendelian randomization analysis

In this study, we conducted a two-sample Mendelian randomization analysis, utilizing random effects inverse-variance weighted (IVW), weighted median, and Mr-Egger methods to evaluate the possible causal relationship between calcium and sleep

disorders by OR value. Since the traditional inverse variance weighted analysis method may suffer from invalid instrument bias or pleiotropy, a sensitivity analysis was conducted to evaluate the robustness and validity of the IVW results obtained.

In an effort to minimize bias caused by horizontal pleiotropy, a phenomenon that influences results through causal pathways rather than exposure, our study employed Mr-PRESSO to detect horizontal pleiotropy in all SNPs. In addition, several other established MR methods, including weighted median and Mr-Egger regression, were employed to further examine the association between calcium and sleep disorder. To ensure the robustness of the findings, statistically significant results were subjected to heterogeneity tests, such as Mr-Egger intercept test, sensitivity analysis, and modified Cochran Q statistic. All statistical tests were two-tailed, and a p-value of less than 0.05 was considered statistically significant. Additionally, we conducted a "leave-one-out" analysis to explore the likelihood of a single SNP driving the causal association.

2.4. NHANES cohort study

2.4.1. Participants

Data from the National Health and Nutrition Examination Survey (NHANES, https://www.cdc.gov/nchs/nhanes/index.htm), spanning from 2007 to 2018, was analyzed in this study. Figure S1 provides an overview of the study design, sampling, and exclusion criteria. Initially, a total of 54,896 participants who were eligible for sleep questionnaires were included. Following the exclusion of individuals with missing data on any of the measures and covariates, the final study sample consisted of 30,837 individuals. Since the sleep questionnaire was administered exclusively to individuals aged 16 and above, the proportion of teenagers in the sample was limited to 4.33%. The distribution of participants across genders was approximately equal, with sex ratio of about 1:1 (Table S2).

2.4.2. Serum Calcium

The Indirect Ion Selective Electrode (I.S.E.) method is employed to measure calcium concentration in serum. This technique works by measuring the activity of free calcium ions in the sample solution. When the sample buffer mixture comes into contact with the electrode, the calcium ions bind with the ionophore at the electrode's surface, generating a change in potential. The resulting measure of total calcium ion units is given in mmol/l.

2.4.3. Total 25-Hydroxyvitamin D in Serum

The ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) is used to measure the concentrations of 25-hydroxyvitamin D3 (25OHD3), epi-25-hydroxyvitamin D3 (epi-25OHD3), and 25-hydroxyvitamin D2 (25OHD2) in human serum. Serum samples are treated by liquid-liquid extraction, PFP column separation, and MS-MS detection, followed by quantitation and calculations.

Total 25-hydroxyvitamin D is the sum of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3, excluding epi-25-hydroxy. Details of the experiment are available at the Laboratory Method Files section for the detailed laboratory procedure manual (NHANES 2007-2008: Vitamin D Data Documentation, Codebook, and Frequencies (cdc.gov)).

2.4.4. sleep disorder

Participants were assessed for sleep disorders using the Sleep Disorder Questionnaire, which asked whether they had ever informed a doctor or healthcare provider about any sleep difficulties [14,15]. Responses of "Yes" or "No" were categorized accordingly, while responses of "Do not know" and "Refused" were considered missing.

2.4.5. Covariates

In this study, age, gender and race were recorded in years at the time of the screening interview. Ethnicity was categorized into five groups: Mexican Americans, other Hispanics, non-Hispanic whites, non-Hispanic blacks, and other ethnic/racial groups (referenced as "others"). Body Mass Index (BMI) was determined by dividing an individual's weight in kilograms (kg) by the square of their standing height in meters (m).

Annual Family Income was regrouped into three categories: Under $20,000, $20,000-$100,000 and Over $100,000. Physical activity collected by physical activity questionnaire which is based on the Global Physical Activity Questionnaire (GPAQ) and includes questions related to daily activities, leisure time activities, and sedentary activities. In this study, we defined physical activity using metabolic equivalents of task (MET), which has been correlated with health benefits in previous research and has also been employed in cohort studies[16,17]. The value of 600 metabolic equivalents of task (MET) min/week was treated as the cut-off point for physical activity inactive or active.

2.4.6. statistical analysis

In line with the Centers for Disease Control and Prevention (CDC) guidelines, analyses were conducted using data from the National Health and Nutrition Examination Survey (NHANES). The sample-weighted results of serum calcium and total 25-Hydroxyvitamin D measurements were analyzed in a quarter of the participants aged 16-18 years. The difference in percentages of subgroup of calcium and vitamin D to sleep disorder phenotypes among survey cycles were tested using the Chi-Squared Test. In Model I, no covariate was considered. In Model II, sociodemographic (sex, age, ethnicity) was considered as covariate. In Model III, sociodemographic and BMI were considered as covariates. In Model IV, sociodemographic, BMI, income and Physical activity were adjusted as covariates.

3. Results

3.1. Instrumental variables for Mendelian randomization

To establish the instrument variables (IVs) for our study, we selected 163 independent SNPs associated with calcium at genome-wide significance (as indicated in Table 1 and Figure 1) from GWASs. After removal of SNPs in linkage disequilibrium, 160 SNPs demonstrated a positive association with sleep disorders, albeit some of them lacked statistical significance. After removing outlier SNPs with MR-PRESSO, 148 SNPs remained as instrumental variables (with a number of bootstrap replicas set at 10,000). The P-value of 1.00E-05 corresponds to an F statistic of approximately 20 [18]. It is worth mentioning that a threshold of $F < 10$ is often employed to characterize "weak IVs." In this study, since our criteria for SNP selection was set at a P-value less than 5.00E-08, the possibility of weak instrument bias was deemed negligible.

3.2. Mendelian randomization results

The influence of each SNP locus on sleep disorders was obtained through Mendelian randomization of two samples, and the results are shown in Figure 1. Our study found evidence supporting a causal association between calcium and sleep disorders using the IVW method (OR, 0.924, 95%CI, 0.855 - 0.998, P, 0.0457), other MR methods showed similar trends, although not statistically significant (Figure 1 and Figure 2). To assess the robustness of our findings, we performed further tests using Mr-Egger and Mr-PRESSO for the included SNPs, and did not detect potential horizontal pleiotropy (P = 0.695). The funnel plot analysis also revealed no bias in our study (Figure 3).

3.3. Heterogeneity and sensitivity test

The modified Cochran Q statistic indicated no significant heterogeneity in the effects of the included SNPs (P = 0.24). We further performed a leave-one-out sensitivity analysis to evaluate the impact of individual SNP sites on the overall causal relationship. As shown in Figure 4, the causal relationship remained unchanged and significant when each SNP was systematically removed and the MR analysis was repeated, indicating that our estimated effect was not driven by a single SNP.

3.4. NHANES

Table S1 outlines the baseline characteristics of participants across survey waves. A study found that the prevalence of sleep disorder was 24.53%, showing significance increase since 2009 ($p < 0.0001$), suggests that the incidence of sleep disorders appears to be increasing annually. To better describe the level of serum calcium and 25-hydroxyvitamin D concentration, serum calcium and 25-hydroxyvitamin D were divided into four intervals by the quartile method. The threshold of serum calcium (mmol/L) concentration is as follows: [1.6,2.3] Q1, (2.3,2.35] Q2, (2.35,2.4] Q3, (2.4,3.7] Q4. The threshold of serum 25-hydroxyvitamin D (nmol/L) concentration is as follows: [6.15,44.3] Q1, (44.3,61.1] Q2, (61.1,79.4] Q3, (79.4,422] Q4. Similarly, we used the quartile method to divide BMI into the following four levels, Q1: [13.18,24], Q2: (24,27.8], Q3: (27.8,32.5], Q4: (32.5,86.2].

Participants were divided into with sleep disorder and without sleep disorder group. The characteristic of participants without sleep disorder and with sleep disorder were presented in Table S2. The analysis indicates that the distribution of sleep disorders in age, race, family income, BMI, serum calcium and 25-hydroxyvitamin D significantly different, indicating that these may affect the occurrence of sleep disorders.

The results of binary logistic regression models of serum trace metals and serum trace metals ratios on self-reported sleep disorders were described in Table 2. Model 1 served as the baseline model with no covariates. Control variables (sex, age, race, BMI and income) were selected using stepwise regression and added to create Model II.

To further investigate the effects of serum calcium on sleep at different concentrations, stratified regression was used. (Table 3). Calcium were likely to be protected factor for sleep disorder in male, black and Physical active populations.

4. Discussion

The study suggests no significant correlation between serum calcium levels and sleep disorders in non-exercising populations. Previous research has suggested a potential protective role of calcium in sleep disorders. For instance, Alkhatatbeh et al.[19] found low calcium intake among university students with sleep disorders, while Grandner et al. [11] demonstrated that calcium intake could improve sleep quality. Jeon et al. [20] identified an association between lower serum calcium levels and disrupted sleep and rest activity rhythms in shift workers. However, these studies have mainly focused on specific populations predominantly adults. Grandner's study, for example, included individuals with an average age of 46.3 years, while Alkhatatbeh's study targeted individuals aged 18-29. The inclusion of different populations can introduce confounding variables and result in inconsistent or inconclusive findings. To systematically evaluate the causal relationship between calcium and sleep disorder, we employed a Mendelian randomization approach. Genetic variation remains stable throughout an individual's life, and alleles are randomly classified and fixed. Hence, so the results of this study provides insights into the potential lifetime benefits of calcium in reducing sleep disorders while avoid bias due to confounding factors and reverse causality [13].

Our analysis of the NHANES cohort revealed an inverse association between calcium levels and sleep disorders among the black, physical active and male population. Notably, this association was not observed among adolescents and physical inactive population. These findings suggest that the relationship between calcium levels and sleep disorders may be influenced by specfic demographic factors. However, further well-designed prospective studies and large-sample randomized controlled trials are needed to determine whether the relationship between calcium levels and sleep is causal or a concomitant phenomenon in physical active

population. In addition, the mechanisms underlying calcium's protective effect on sleep disorders remain unclear. Previous study have implicated calcium's role in regulating the generation of slow-wave sleep and its impact on sleep rhythms through the calcium channel (Ca(V)3.3) in the thalamus [20–22]. Notably, the use of calcium channel blockers has been shown to significantly decrease total sleep time ($p = 0.037$) [23]. Although progress has been made in understanding these mechanisms, current knowledge is limited to specific populations[20,21,23] and certain animal models [22]. Therefore, further research is needed to fully comprehend these mechanisms and their broader implications.

There are several limitations to our study. One potential limitation the exclusive inclusion of populations of European descent in the GWAS studies, which lacks ethnic diversity. Therefore, it is unclear whether there are genetic differences among various ethnic groups, countries, and regions. Furthermore, the lack of comprehensive clinical information prevented subgroup analysis and determination of specific causal associations. To address these limitations, we incorporated data from the NHANES population in our study. However, it should be noted that the use of questionnaire data to assess sleep disorders in NHANES may be influenced by human bias, potentially affecting the accuracy and reliability of the results. Additionally, as sleep and circadian rhythms involve complex stages, our study did not segment the sleep process to examine which aspect of sleep are affected by serum calcium levels. Future research that involves objective sleep measurements could advance our understanding in this area. Despite these limitations, our study provides valuable insights into the relationship between calcium and sleep disorders, offering guidance on the use of calcium supplements.

5. Conclusions

Our findings indicate that there is no notable correlation between calcium levels and sleep disorders among individuals who do not engage in regular exercise. It is important to exercise caution when considering long-term high-dose calcium supplementation in clinical practice. It may be necessary to tailor calcium supplementation strategies for diverse populations based on their individual

needs. Exercise may be an important mediator in the relationship between sleep and calcium levels. Further studies on the relationship between sleep disorders and calcium levels in different populations can be conducted in the future to further provide calcium supplement recommendations for different populations in the clinic.

6. Author Contributions

Conceptualization, L.M. and J.C.; methodology, L.M., B.T and Y.H.; software, L.M.; validation, T.P., Z.L. and L.C.; formal analysis, L.M. and B.T.; writing—original draft preparation, L.M. and W.D; writing—review and editing, L.M and D.W; supervision, J.C. and W.D. All authors have read and agreed to the published version of the manuscript.

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8. Conflicts of Interest

The authors declare no conflict of interest.

9. Ethical statement

The data used in this study was obtained from NHANES, UK Biobank and FinnGen, which provides publicly available data for research purposes. The data used in this study was obtained from public databases and no personal identifying information of the participants was included. The findings presented in this study contribute to scientific knowledge and can inform policy decisions that benefit public health.

References

1. Anafi, R.C.; Kayser, M.S.; Raizen, D.M. Exploring Phylogeny to Find the Function of Sleep. *Nat. Rev. Neurosci.* **2019**, *20*, 109–116, doi:10.1038/s41583-018-0098-9.

2. Mason, G.M.; Lokhandwala, S.; Riggins, T.; Spencer, R.M.C. Sleep and Human Cognitive Development. *Sleep Med. Rev.* **2021**, *57*, 101472, doi:10.1016/j.smrv.2021.101472.

3. Rasch, B.; Born, J. About Sleep's Role in Memory. *Physiol. Rev.* **2013**, *93*, 681–766, doi:10.1152/physrev.00032.2012.

4. Holmer, B.J.; Lapierre, S.S.; Jake-Schoffman, D.E.; Christou, D.D. Effects of Sleep Deprivation on Endothelial Function in Adult Humans: A Systematic Review. *GeroScience* **2021**, *43*, 137–158, doi:10.1007/s11357-020-00312-y.

5. Vitiello, M.V.; Bliwise, D.L.; Prinz, P.N. Sleep in Alzheimer's Disease and the Sundown Syndrome. *Neurology* **1992**, *42*, 83–93; discussion 93-94.

6. Besedovsky, L.; Lange, T.; Haack, M. The Sleep-Immune Crosstalk in Health and Disease. *Physiological reviews* **2019**, *99*, 1325–1380, doi:10.1152/physrev.00010.2018.

7. Madrid-Valero, J.J.; Gregory, A.M. Behaviour Genetics and Sleep: A Narrative Review of the Last Decade of Quantitative and Molecular Genetic Research in Humans. *Sleep Med. Rev.* **2023**, *69*, 101769, doi:10.1016/j.smrv.2023.101769.

8. Thompson, R.S.; Gaffney, M.; Hopkins, S.; Kelley, T.; Gonzalez, A.; Bowers, S.J.; Vitaterna, M.H.; Turek, F.W.; Foxx, C.L.; Lowry, C.A.; et al. Ruminiclostridium 5, Parabacteroides Distasonis, and Bile Acid Profile Are Modulated by Prebiotic Diet and Associate with Facilitated Sleep/Clock Realignment after Chronic Disruption of Rhythms. *Brain, Behavior, and Immunity* **2021**, *97*, 150–166, doi:10.1016/j.bbi.2021.07.006.

9. Ikonte; Mun; Reider; Grant; Mitmesser Micronutrient Inadequacy in Short Sleep: Analysis of the NHANES 2005–2016. *Nutrients* **2019**, *11*, 2335, doi:10.3390/nu11102335.

10. Alkhatatbeh, M.J.; Abdul-Razzak, K.K.; Khwaileh, H.N. Poor Sleep Quality among Young Adults: The Role of Anxiety, Depression, Musculoskeletal Pain, and Low Dietary Calcium Intake. *Perspect. Psychiatr. Care* **2021**, *57*, 117–128, doi:10.1111/ppc.12533.

11. Grandner, M.A.; Jackson, N.; Gerstner, J.R.; Knutson, K.L. Sleep Symptoms Associated with Intake of Specific Dietary Nutrients. *J Sleep Res* **2014**, *23*, 22–34, doi:10.1111/jsr.12084.

12. Smith, G.D.; Ebrahim, S. "Mendelian Randomization": Can Genetic Epidemiology Contribute to Understanding Environmental Determinants of Disease? *Int. J. Epidemiol.* **2003**, *32*, 1–22, doi:10.1093/ije/dyg070.

13. Smith, G.D.; Lawlor, D.A.; Harbord, R.; Timpson, N.; Day, I.; Ebrahim, S. Clustered Environments and Randomized Genes: A Fundamental Distinction between Conventional and Genetic Epidemiology. *PLoS medicine* **2007**, *4*, e352, doi:10.1371/journal.pmed.0040352.

14. Prather, A.A.; Leung, C.W. Association of Insufficient Sleep With Respiratory Infection Among Adults in the United States. *JAMA Intern Med* **2016**, *176*, 850–852, doi:10.1001/jamainternmed.2016.0787.

15. Centers for Disease Control and Prevention (CDC) Effect of Short Sleep Duration on Daily Activities--United States, 2005-2008. *MMWR Morb Mortal Wkly Rep* **2011**, *60*, 239–242.

16. Deng, M.-G.; Liu, F.; Liang, Y.; Chen, Y.; Nie, J.-Q.; Chai, C.; Wang, K. Associations of Serum Zinc, Copper, and Selenium with Sleep Disorders in the American Adults: Data from NHANES 2011–2016. *Journal of Affective Disorders* **2023**, *323*, 378–385, doi:10.1016/j.jad.2022.11.088.

17. Piercy, K.L.; Troiano, R.P.; Ballard, R.M.; Carlson, S.A.; Fulton, J.E.; Galuska, D.A.; George, S.M.; Olson, R.D. The Physical Activity Guidelines for Americans. *JAMA* **2018**, *320*, 2020, doi:10.1001/jama.2018.14854.

18. Burgess, S.; Butterworth, A.; Thompson, S.G. Mendelian Randomization Analysis with Multiple Genetic Variants Using Summarized Data. *Genet. Epidemiol.* **2013**, *37*, 658–665, doi:10.1002/gepi.21758.

19. Alkhatatbeh, M.J.; Hmoud, Z.L.; Abdul-Razzak, K.K.; Alem, E.M. Self-Reported Sleep Bruxism Is Associated with Vitamin D Deficiency and Low Dietary Calcium Intake: A Case-Control Study. *BMC oral health* **2021**, *21*, 21, doi:10.1186/s12903-020-01349-3.

20. Jeon, Y.-S.; Yu, S.; Kim, C.; Lee, H.J.; Yoon, I.-Y.; Kim, T. Lower Serum Calcium Levels Associated with Disrupted Sleep and Rest–Activity Rhythm in Shift Workers. *Nutrients* **2022**, *14*, 3021, doi:10.3390/nu14153021.

21. Tatsuki, F.; Sunagawa, G.A.; Shi, S.; Susaki, E.A.; Yukinaga, H.; Perrin, D.; Sumiyama, K.; Ukai-Tadenuma, M.; Fujishima, H.; Ohno, R.; et al. Involvement of Ca(2+)-Dependent Hyperpolarization in Sleep Duration in Mammals. *Neuron* **2016**, *90*, 70–85, doi:10.1016/j.neuron.2016.02.032.

22. Astori, S.; Wimmer, R.D.; Prosser, H.M.; Corti, C.; Corsi, M.; Liaudet, N.; Volterra, A.; Franken, P.; Adelman, J.P.; Lüthi, A. The Ca(V)3.3 Calcium Channel Is the Major Sleep Spindle Pacemaker in Thalamus. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 13823–13828, doi:10.1073/pnas.1105115108.

23. Nerbass, F.B.; Pedrosa, R.P.; Genta, P.R.; Drager, L.F.; Lorenzi-Filho, G. Calcium Channel Blockers Are Independently Associated with Short Sleep Duration in Hypertensive Patients with Obstructive Sleep Apnea. *Journal of hypertension* **2011**, *29*, 1236–1241, doi:10.1097/HJH.0b013e3283462e8b.

Figures, Tables and Schemes

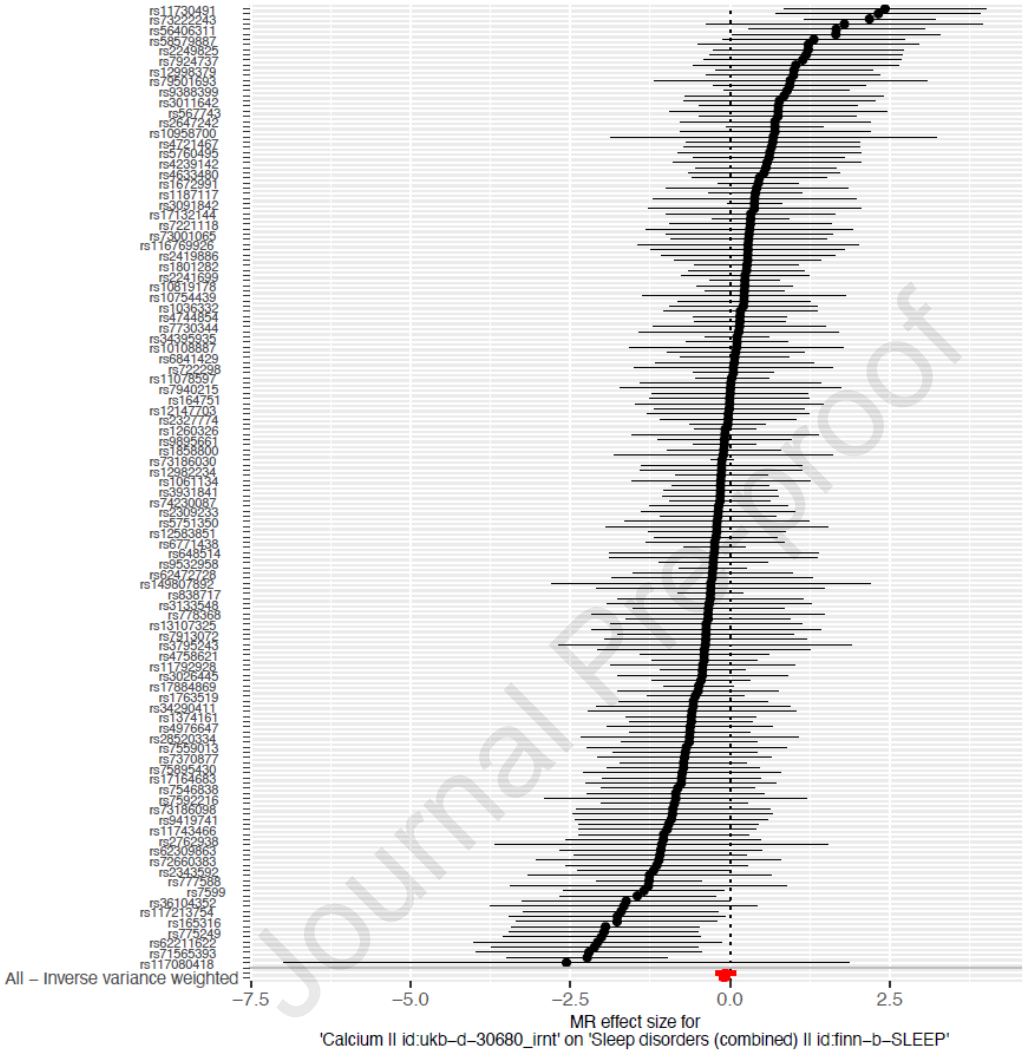


Figure 1：Forest plot of the causal effects of SNP associated with calcium on sleep disorders. The red lines represent the MR results obtained from the MR-Egger test and IVW method.

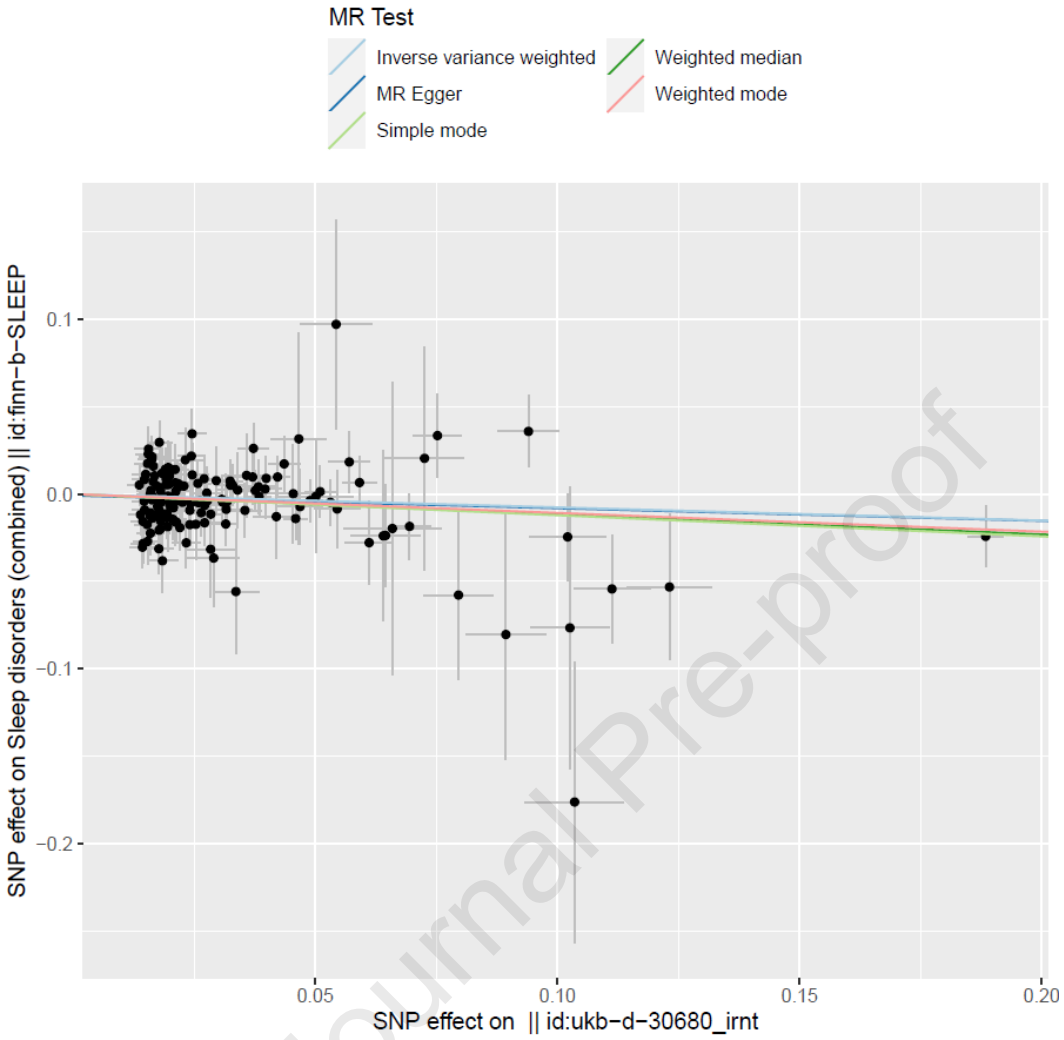


Figure 2. Scatter plots of genetic associations with body mass index against the genetic associations with sleep disorders. Each line represent the causal association for each method.

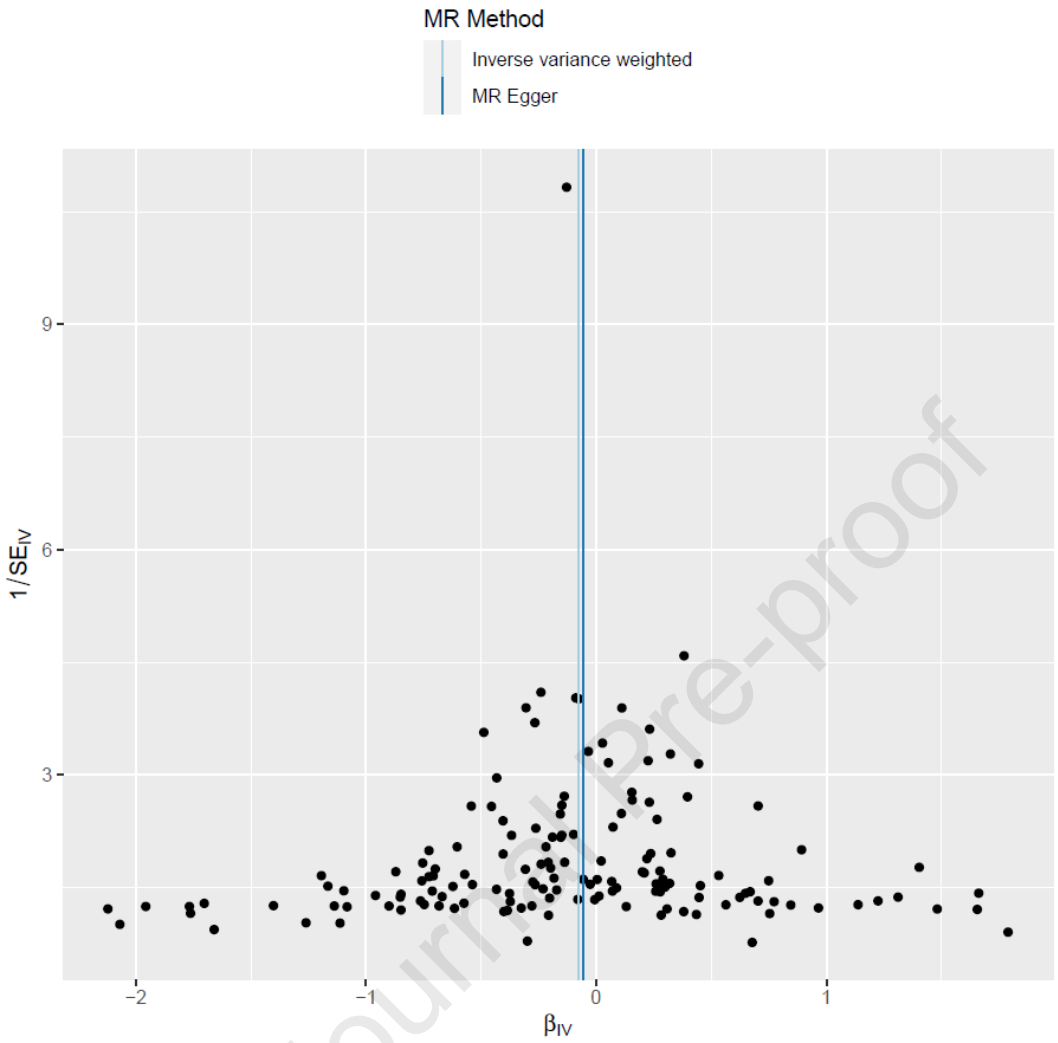


Figure 3. Funnel plot to assess heterogeneity. The blue line represents the inverse-variance weighted estimate, and the dark blue line represents the Mendelian Randomization-Egger estimate.

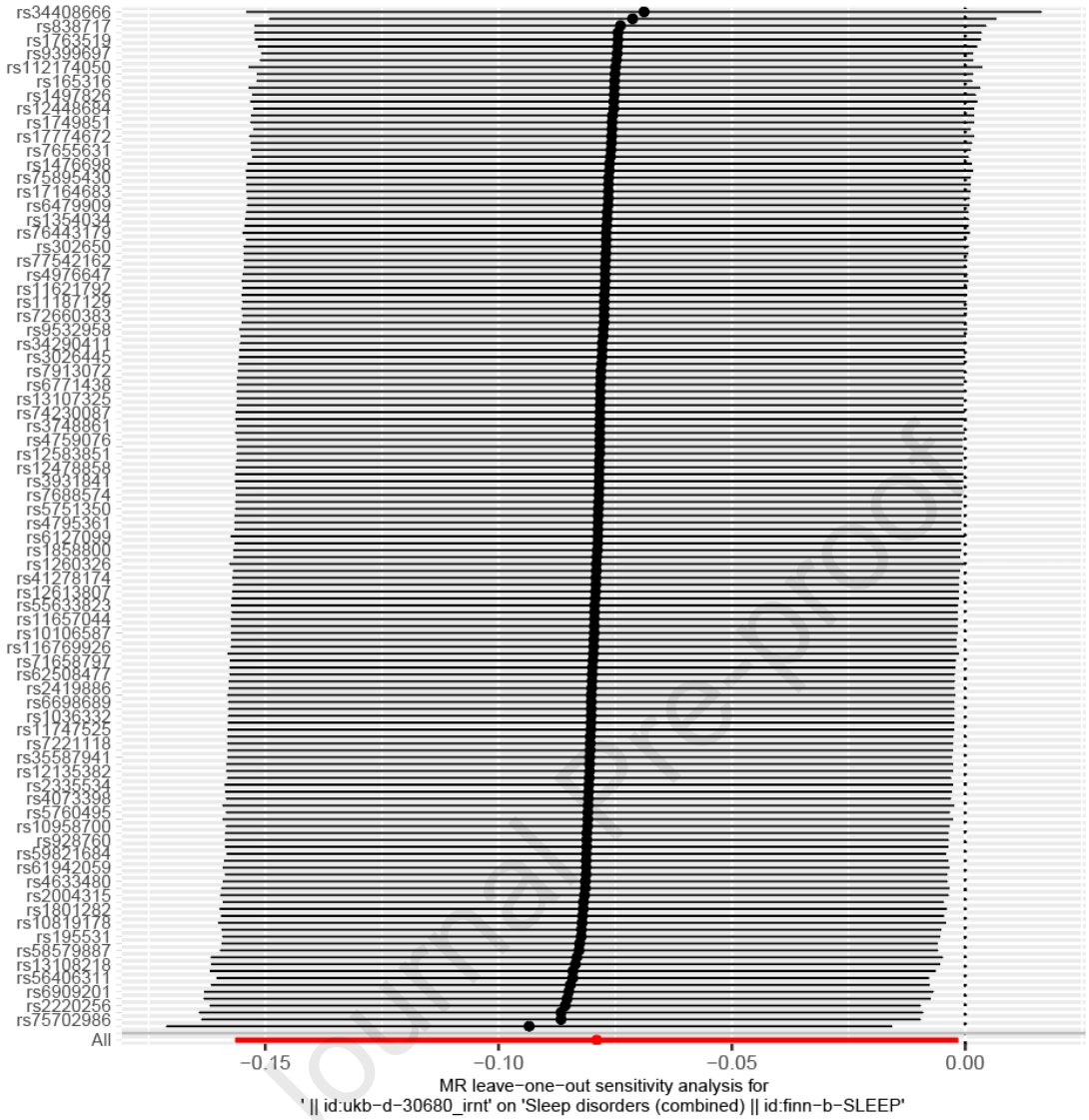


Figure 4. Result of "leave-one out" sensitive analysis.

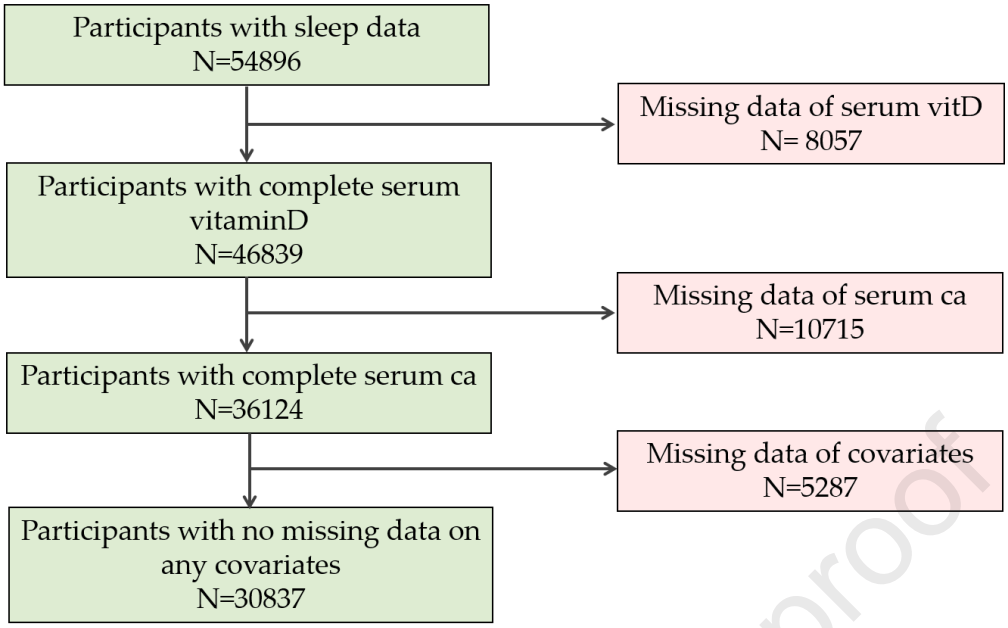


Figure s1. workflow of the population included in our final analysis.

Table 1. Summary of the GWAS included in this study

Variable	ID	Number of SNPs	Race	Sex	Year	Sample Size
Calcium	ukb-d-30680_irnt	13585309	European	female and male	2018	315153
Sleep disorder	finn-b-SLEEP	16380458	European	female and male	2021	216700

Table S1. Characteristic in sleep disorders of participants across NHANES 2007–2018 cycles.

Variable	Total	2007-2008	2009-2010	2011-2012	2013-2014	2015-2016	2017-2018	P
	n=30837	n=4795	n=5781	n=5009	n=5473	n=5189	n=4590	
Sleep disorder?								< 0.0001
No	23274(75.47)	3738(76.52)	4436(75.82)	3873(74.14)	4093(72.62)	3839(71.07)	3295(68.99)	
Yes	7563(24.53)	1057(23.48)	1345(24.18)	1136(25.86)	1380(27.38)	1350(28.93)	1295(31.01)	

Table S2. Characteristics of participants with and without sleep disorders.

Variable	Total	Without sleep disorder	With sleep disorder	P
	n=30837	23274	7563	
Age				< 0.0001
<18	1335(4.33)	1202(3.44)	133(1.12)	
>=18	29502(95.67)	22072(96.56)	7430(98.88)	
Sex				< 0.0001
Female	15836(51.35)	11409(49.21)	4427(58.28)	
Male	15001(48.65)	11865(50.79)	3136(41.72)	
Race				< 0.0001
Black	6383(20.7)	4868(11.10)	1515(9.50)	
Mexican	4844(15.71)	4003(10.08)	841(5.29)	
Other	6976(22.62)	5583(14.82)	1393(10.51)	
White	12634(40.97)	8820(63.99)	3814(74.70)	
Income				< 0.001
Under $20,00	19089(61.9)	14631(61.30)	4458(59.37)	
$20,000-$100,0	5157(16.72)	3962(25.25)	1195(24.35)	
Over $100,00	6591(21.37)	4681(13.45)	1910(16.29)	
BMI				< 0.0001
Q1	7810(25.33)	6250(26.74)	1560(21.53)	
Q2	7656(24.83)	6025(26.18)	1631(22.42)	
Q3	7745(25.12)	5860(25.05)	1885(24.54)	
Q4	7626(24.73)	5139(22.02)	2487(31.51)	
Physical activity				< 0.0001
Active	18648(60.47)	14421(66.53)	4227(59.89)	
Inactive	12189(39.53)	8853(33.47)	3336(40.11)	
Vitamin D				< 0.0001
Q1	7732(25.07)	6040(18.26)	1692(16.22)	
Q2	7677(24.9)	5975(23.24)	1702(20.43)	
Q3	7728(25.06)	5913(28.32)	1815(26.20)	
Q4	7700(24.97)	5346(30.19)	2354(37.15)	
Calcium				0.01
Q1	10388(33.69)	7655(32.85)	2733(35.75)	
Q2	7076(22.95)	5361(23.63)	1715(22.83)	
Q3	6282(20.37)	4815(20.89)	1467(19.90)	
Q4	7091(23)	5443(22.63)	1648(21.52)	

* P < 0.05 was considered statistically significant and presented in bold. Abbreviations: BMI, body mass index. (P values were two-tailed and considered statistically significant at P < .05)

Table 2. Associations of serum calcium and Vitamin D with sleep disorder in American adolescence.

Variable	Model I		Model II		Model III		Model IV	
	OR (95% CI)	Pvalue	OR (95% CI)	Pvalue	OR (95% CI)	Pvalue	OR (95% CI)	Pvalue
Calcium								
Q1	ref	ref	ref	ref	ref	ref	ref	ref
Q2	0.89(0.81,0.97)	0.01	0.90(0.82,0.98)	0.01	0.94(0.86,1.02)	0.15	0.94(0.86,1.02)	0.14
Q3	0.88(0.79,0.97)	0.01	0.91(0.82,1.01)	0.07	0.97(0.87,1.08)	0.56	0.97(0.87,1.07)	0.5
Q4	0.87(0.80,0.96)	0.01	0.91(0.83,1.01)	0.07	0.97(0.88,1.08)	0.6	0.96(0.87,1.06)	0.39

OR, odds ratio; 95 % CI, 95 % confidence interval; Model I: No adjustment was made for any covariates. Model II: Adjusted for sociodemographic (sex, age, ethnicity). Model III: Adjusted for sociodemographic and BMI. Model IV: Adjusted for sociodemographic, BMI, income and Physical activity.

Table 3. The results for stratified regression of serum calcium on sleep in subgroups.

Character	Q1	Q2	Q3	Q4	p for trend
Sex					
Female	ref	0.97(0.86,1.08)	0.98(0.87,1.11)	1.04(0.91,1.17)	0.66
Male	ref	0.86(0.73,1.00)	0.87(0.75,1.02)	0.82(0.70,0.95)	0.02
Race					
Other	ref	0.79(0.63,0.99)	0.86(0.67,1.11)	0.82(0.64,1.06)	0.16
White	ref	0.93(0.83,1.05)	0.93(0.82,1.07)	0.92(0.81,1.05)	0.22
Mexican	ref	0.85(0.68,1.08)	0.78(0.63,0.97)	0.82(0.62,1.08)	0.06
Black	ref	0.86(0.71,1.03)	0.79(0.66,0.94)	0.76(0.65,0.89)	<0.001
Physical activity					
Active	ref	0.88(0.79,0.99)	0.87(0.76,1.00)	0.83(0.73,0.95)	0.01
Inactive	ref	0.95(0.83,1.10)	0.99(0.84,1.16)	1.01(0.89,1.14)	0.87
Age					
<18	ref	1.68(0.74,3.85)	1.22(0.61,2.44)	1.51(0.80,2.86)	0.54
>=18	ref	0.91(0.83,1.00)	0.93(0.84,1.02)	0.92(0.83,1.02)	0.1

Table 1. Summary of the GWAS included in this study

Variable	ID	Number of SNPs	Race	Sex	Year	Sample Size
Calcium	ukb-d-30680_irnt	13585309	European	female and male	2018	315153
Sleep disorder	finn-b-SLEEP	16380458	European	female and male	2021	216700

Table S1. Characteristic in sleep disorders of participants across NHANES 2007–2018 cycles

Variable	Total	2007-2008	2009-2010	2011-2012	2013-2014	2015-2016	2017-2018	P
	n=30837	n=4795	n=5781	n=5009	n=5473	n=5189	n=4590	
Sleep disorder?								< 0.0001
No	23274(75.47)	3738(76.52)	4436(75.82)	3873(74.14)	4093(72.62)	3839(71.07)	3295(68.99)	
Yes	7563(24.53)	1057(23.48)	1345(24.18)	1136(25.86)	1380(27.38)	1350(28.93)	1295(31.01)	

Table S2. Characteristics of participants with and without sleep disorders.

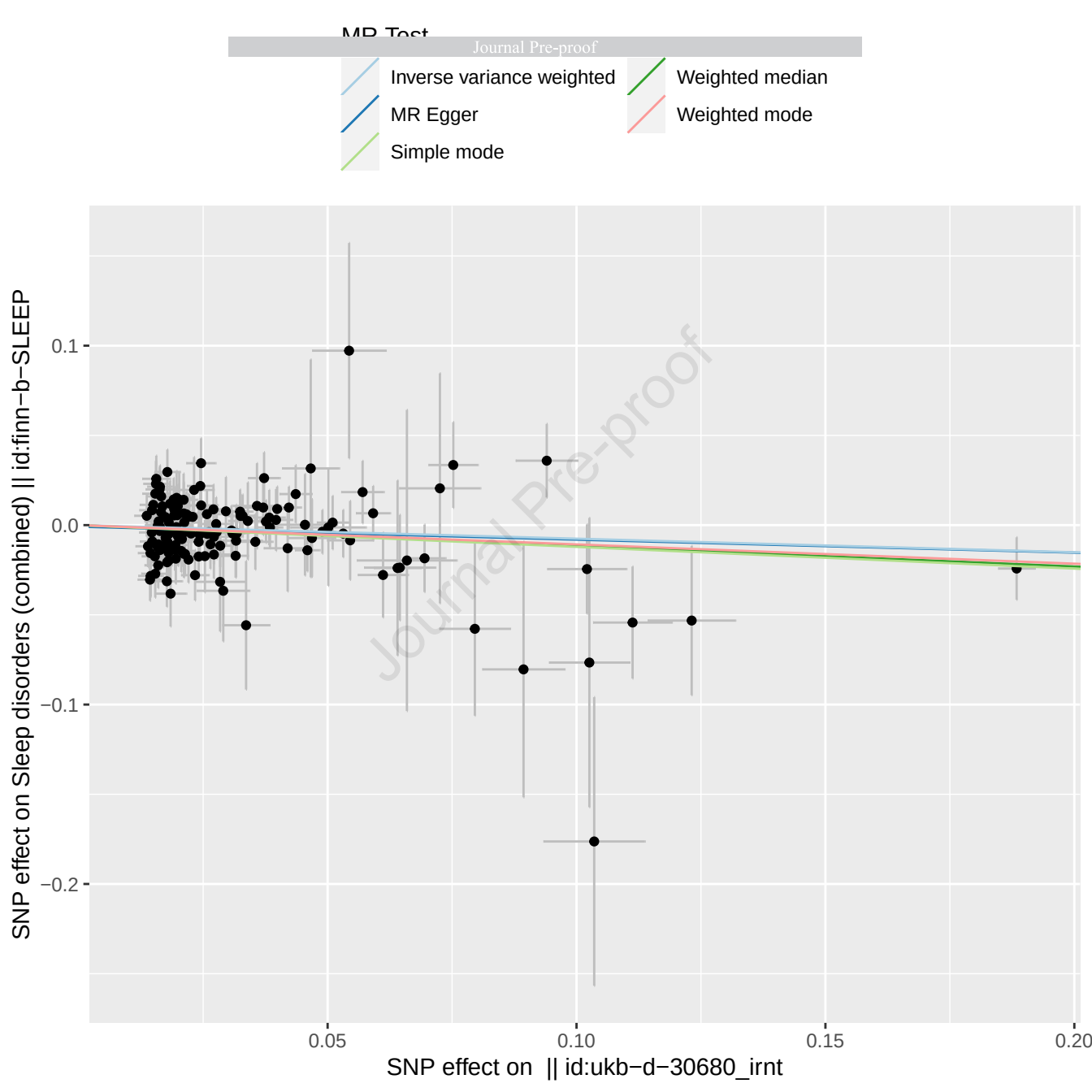
Variable	Total	Without sleep disorder	With sleep disorder	P
	n=30837	23274	7563	
Age				< 0.0001
<18	1335(4.33)	1202(3.44)	133(1.12)	
>=18	29502(95.67)	22072(96.56)	7430(98.88)	
Sex				< 0.0001
Female	15836(51.35)	11409(49.21)	4427(58.28)	
Male	15001(48.65)	11865(50.79)	3136(41.72)	
Race				< 0.0001
Black	6383(20.7)	4868(11.10)	1515(9.50)	
Mexican	4844(15.71)	4003(10.08)	841(5.29)	
Other	6976(22.62)	5583(14.82)	1393(10.51)	
White	12634(40.97)	8820(63.99)	3814(74.70)	
Income				< 0.001
Under $20,000	19089(61.9)	14631(61.30)	4458(59.37)	
$20,000-$100,00	5157(16.72)	3962(25.25)	1195(24.35)	
Over $100,000	6591(21.37)	4681(13.45)	1910(16.29)	
BMI				< 0.0001
Q1	7810(25.33)	6250(26.74)	1560(21.53)	
Q2	7656(24.83)	6025(26.18)	1631(22.42)	
Q3	7745(25.12)	5860(25.05)	1885(24.54)	
Q4	7626(24.73)	5139(22.02)	2487(31.51)	
Physical activity				< 0.0001
Active	18648(60.47)	14421(66.53)	4227(59.89)	
Inactive	12189(39.53)	8853(33.47)	3336(40.11)	
Vitamin D				< 0.0001
Q1	7732(25.07)	6040(18.26)	1692(16.22)	
Q2	7677(24.9)	5975(23.24)	1702(20.43)	
Q3	7728(25.06)	5913(28.32)	1815(26.20)	
Q4	7700(24.97)	5346(30.19)	2354(37.15)	
Calcium				0.01
Q1	10388(33.69)	7655(32.85)	2733(35.75)	
Q2	7076(22.95)	5361(23.63)	1715(22.83)	
Q3	6282(20.37)	4815(20.89)	1467(19.90)	
Q4	7091(23)	5443(22.63)	1648(21.52)	

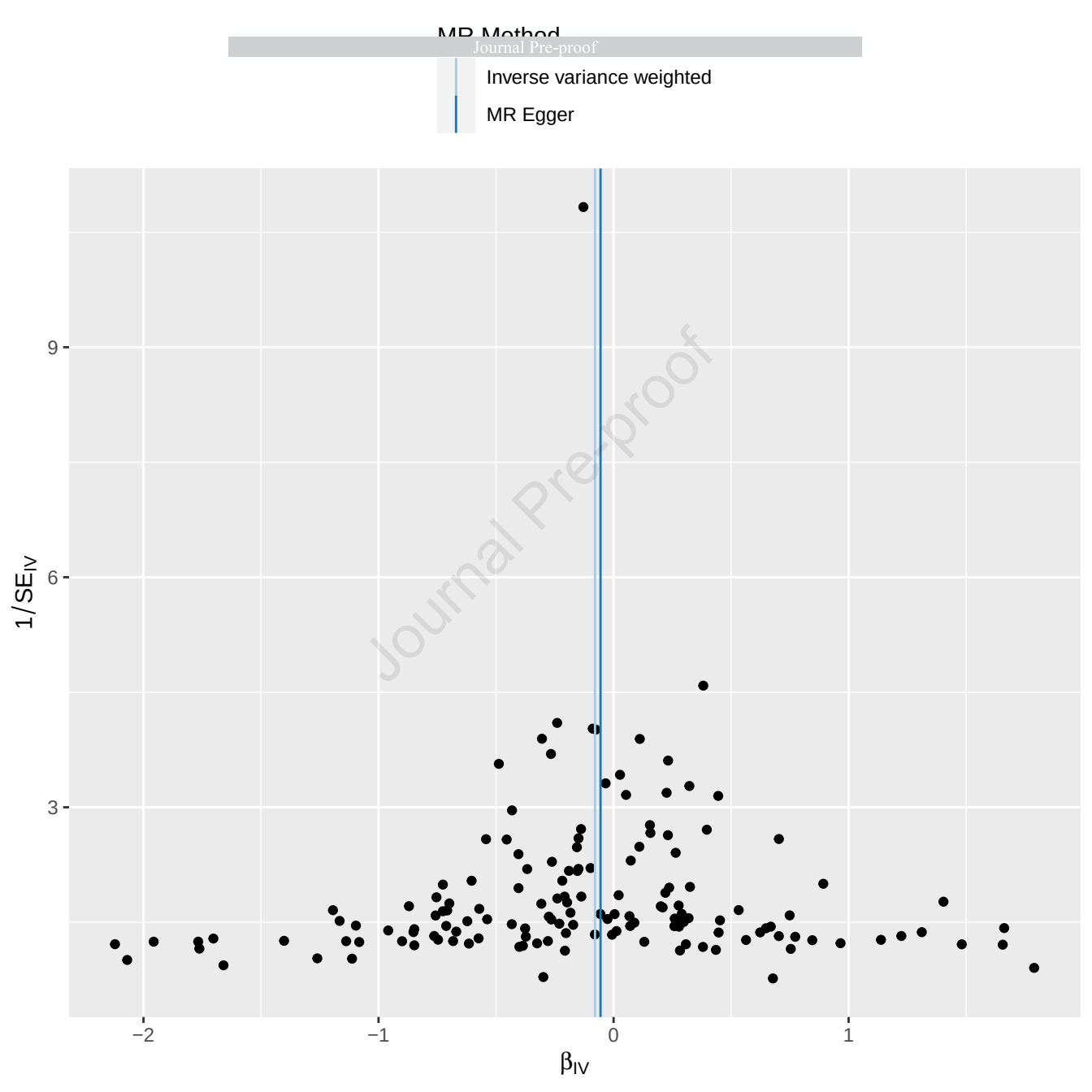
Table 2. Associations of serum calcium and Vitamin D with sleep disorder in American adolescence.

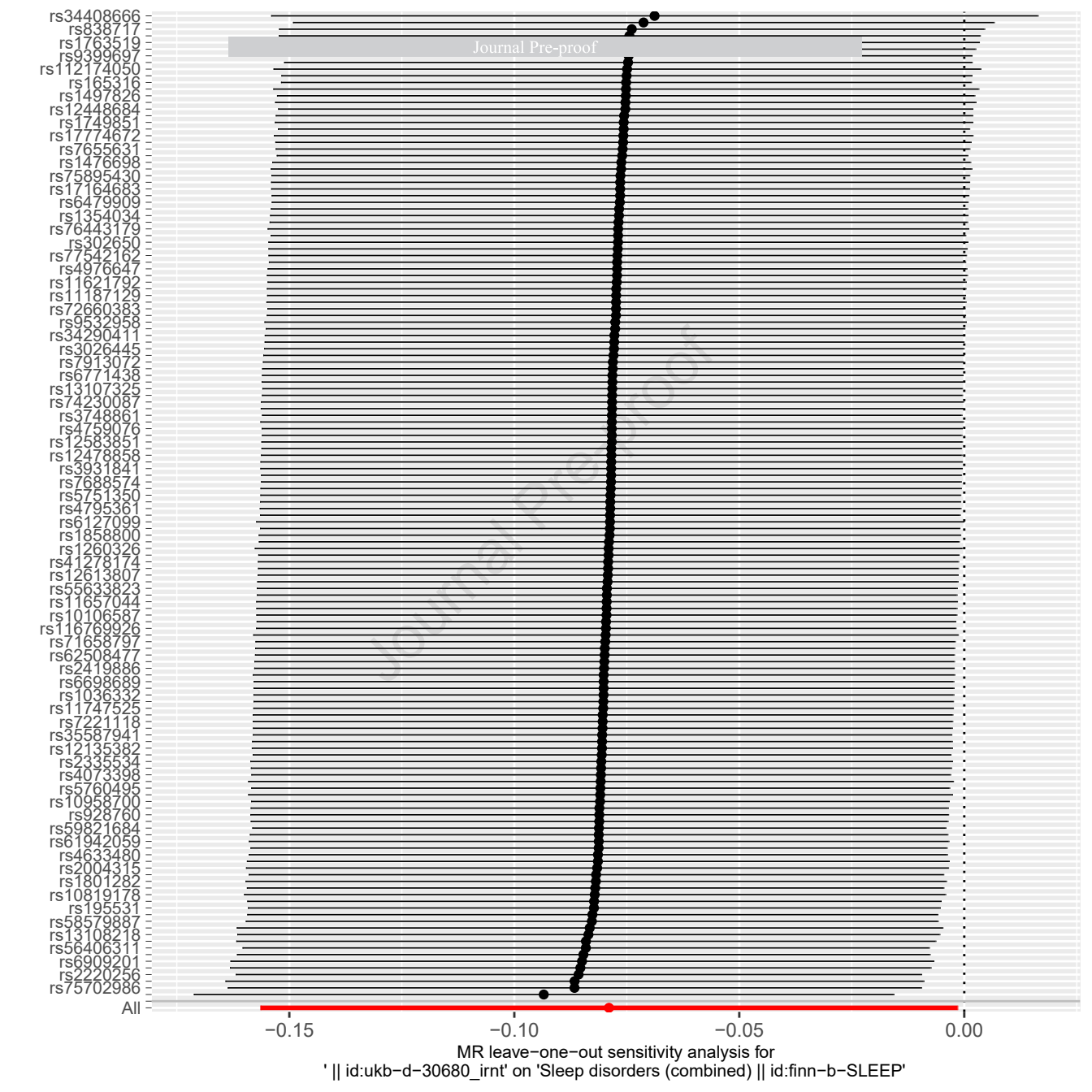
Variable	Model I		Model II		Model III		Model IV	
	OR (95% CI)	Pvalue	OR (95% CI)	Pvalue	OR (95% CI)	Pvalue	OR (95% CI)	Pvalue
Calcium								
Q1	ref	ref	ref	ref	ref	ref	ref	ref
Q2	0.89(0.81,0.97)	0.01	0.90(0.82,0.98)	0.01	0.94(0.86,1.02)	0.15	0.94(0.86,1.02)	0.14
Q3	0.88(0.79,0.97)	0.01	0.91(0.82,1.01)	0.07	0.97(0.87,1.08)	0.56	0.97(0.87,1.07)	0.5
Q4	0.87(0.80,0.96)	0.01	0.91(0.83,1.01)	0.07	0.97(0.88,1.08)	0.6	0.96(0.87,1.06)	0.39

Table 3. The results for stratified regression of serum calcium on sleep in subgroups.

Character	Q1	Q2	Q3	Q4	p for trend
Sex					
Female	ref	0.97(0.86,1.08)	0.98(0.87,1.11)	1.04(0.91,1.17)	0.66
Male	ref	0.86(0.73,1.00)	0.87(0.75,1.02)	0.82(0.70,0.95)	0.02
Race					
Other	ref	0.79(0.63,0.99)	0.86(0.67,1.11)	0.82(0.64,1.06)	0.16
White	ref	0.93(0.83,1.05)	0.93(0.82,1.07)	0.92(0.81,1.05)	0.22
Mexican	ref	0.85(0.68,1.08)	0.78(0.63,0.97)	0.82(0.62,1.08)	0.06
Black	ref	0.86(0.71,1.03)	0.79(0.66,0.94)	0.76(0.65,0.89)	<0.001
Physical activity					
Active	ref	0.88(0.79,0.99)	0.87(0.76,1.00)	0.83(0.73,0.95)	0.01
Inactive	ref	0.95(0.83,1.10)	0.99(0.84,1.16)	1.01(0.89,1.14)	0.87
Age					
<18	ref	1.68(0.74,3.85)	1.22(0.61,2.44)	1.51(0.80,2.86)	0.54
>=18	ref	0.91(0.83,1.00)	0.93(0.84,1.02)	0.92(0.83,1.02)	0.1



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