Genetically predicted insomnia and lung cancer risk: a Mendelian randomization study

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PII: S1389-9457(21)00444-5

DOI: https://doi.org/10.1016/j.sleep.2021.06.044

Reference: SLEEP 5058

To appear in: *Sleep Medicine*

Received Date: 4 April 2021

Revised Date: 13 June 2021

Accepted Date: 14 June 2021

Please cite this article as: Huo Z, Ge F, Li C, Cheng H, Lu Y, Wang R, Wen Y, Yue K, Pan Z, Peng H, Wu X, Liang H, He J, Liang W, Genetically predicted insomnia and lung cancer risk: a Mendelian randomization study, *Sleep Medicine*, https://doi.org/10.1016/j.sleep.2021.06.044.

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ABSTRACT

Background:

The relationship between insomnia and lung cancer is scanty. The Mendelian randomization approach provides the rationale for evaluating the potential causality between genetically-predicted insomnia and lung cancer risk.

Methods:

We extracted 148 insomnia-related single-nucleotide polymorphisms (SNPs) as instrumental variables (IVs) from published genome-wide association studies (GWASs). Summary data of individual-level genetic information of participants were obtained from the International Lung Cancer Consortium (ILCCO) (29,266 cases and 56,450 controls). MR analyses were performed using the inverse-variance-weighted approach, MR pleiotropy residual sum and outlier (MR-PRESSO) test, weighted median estimator, and MR-Egger regression. Sensitivity analyses were further performed using Egger intercept analysis, leave-one-out analysis, MR-PRESSO global test, and Cochran's Q test to verify the robustness of our findings.

Results:

The results of the MR analysis indicated an increased risk of lung cancer in insomnia patients (OR = 1.1671; 95% CI 1.0754–1.2666, p = 0.0002). The subgroup analyses showed increased risks of lung adenocarcinoma (OR = 1.1878; 95% CI 1.0594–1.3317, p = 0.0032) and squamous cell lung cancer (OR = 1.1595; 95% CI 1.0248–1.3119, p = 0.0188).

Conclusion:

Our study indicated that insomnia is a causal risk factor in the development of lung cancer. Due to the lack of evidence on both the epidemiology and the mechanism level, more studies are needed to better elucidate the results of the study.

Keywords: Insomnia; Lung cancer; Mendelian randomization; Genetics

INTRODUCTION

Characterized by frequent and persistent difficulties falling asleep or difficulties with sleep maintenance that lead to poor sleep satisfaction, insomnia is a highly prevalent condition across the world[1]. Due to the heterogeneity in the definitions, inconsistencies in the diagnostic criteria, and the lack of a more diverse survey population of insomnia, there is considerable variation in epidemiological studies regarding the prevalence of insomnia. Previous studies have shown that more than 30% of adults currently have symptoms of insomnia disorder, in which up to 50% of the subjects are older than 65 years old [2,3].

Although the relationship between sleep traits and lung cancer has been investigated, most of the studies were reflected in poor sleep habits like prolonged or shortened sleep duration and reported inconsistent results of cancer incidence[4-8]. Previous studies have revealed the association between short sleep duration and reduced immunity which is reflected by increased levels of tumor necrosis factor-α (TNF-α) in peripheral blood circulation in sleep-deprived models and reduced numbers of natural killer (NK) in humans[9,10]. However, insomnia disorder is not simply characterized by reduced sleep duration but more by sleep disturbance due to subjective volitional abnormalities. Epidemiology evidence had well demonstrated the increased breast cancer and colorectal cancer risk in insomnia patients, but information on insomnia patients regarding the risk of lung cancer is still scanty.

Recently, Xie et al. [11] conducted a prospective cohort study in UK Biobank and evaluated the relationships between sleep traits and lung cancer risk, which provided the first-ever observational evidence of insomnia-lung cancer relationship. However, due to the limitations of observational

design, the results may be biased by sample size, reverse causality, and potential confounders, leading to the uncertainty of the causal relationships between the exposures and the outcomes. Furthermore, randomized controlled trials (RCTs) are also not suitable for this issue, given the excessive latency between insomnia and lung cancer, the irregularity of insomnia disorder occurrence, and the variation of insomnia complaints. To clarify the insomnia-lung cancer causal relationship could be beneficial for a better interpretation of the causes of lung cancer and the future direction of public health strategy. Therefore, other study designs are urgently needed to evaluate the relationship between insomnia and lung cancer.

Considered as a promising epidemiological approach, the Mendelian randomization (MR) analysis was proposed to precisely evaluate the potential causality between an exposure and an outcome[12,13]. On the basis of Mendel's Second Law, random assortment of alleles results in a random assignment of exposures that are associated with an allele or a set of alleles during the time of gamete formation, which is often independent of environmental risk factors, and precede risk factors and the disease progression[14]. Thus, genetic variants are adopted as the instrumental variables (IVs) in the MR analysis, making it less susceptible to reverse causality and potential confounders. Meanwhile, based on previous genome-wide association studies (GWASs) databases, it is practical to explore the possible relationship and causality of insomnia on lung cancer. In this study, we conducted a two-sample MR analysis to comprehensively explore the potential relationship of insomnia, and other sleep traits, with lung cancer by using the summary data from published GWASs.

METHODS

Genetic Instruments Data for Insomnia and Other Sleep Traits

For the association between genetic variants and insomnia, we extracted available summary data from the published GWAS of the Jansen et al. study[15], from which the outcomes were selected at the genome-wide significance threshold of $p < 5\times10^{-8}$, building up a total of 250 SNPs of European ancestry as the original IVs sample. SNPs of other sleep traits were also extracted from Jansen et al. [15] for subgroup analyses (277 SNPs for morningness, 53 SNPs for sleep duration, 42 SNPs for snoring, and 7 SNPs for snapping); Supplementary table 1 provides detailed information about these SNPs. Data of the Jansen et al. study mainly from the UK-Biobank (UKB) and 23andMe. The majority population of the two databases (UKB: mean age =56.7; 23andMe: two-thirds of the sample older than 45, one-third older than 60 years of age) was more elder people. We performed an exclusion once mutual linkage disequilibrium (LD) analysis shared a larger *p*-value conjugately and surpassed the limited value ($R^2 < 0.001$). Furthermore, several loci were further excluded to eliminate the genetic bias originated from palindrome with intermediate allele frequencies. Ultimately, 148 SNPs were brought into the final IV set as genetic variables (Supplementary table 2). These 148 SNPs explained 1.44% of the variation in insomnia across individuals. The F-statistic was 131.4, which is much larger than 10, implying that the IVs strongly predict the occurrences of insomnia in the samples[16].

GWAS Summary Data on Outcomes

Published genetic summary data on lung cancer from the International Lung Cancer Consortium (ILCCO) (29,266 cases and 56,450 controls from European ancestry, including non-specific lung cancer and its two subtypes: lung adenocarcinoma and squamous cell lung cancer) was obtained for

analysis [17,18] (Table 1). We calculated the $OR_{80\%\ power}$ based on the Burgess et al.[19] study.

We also evaluated the causality between insomnia and breast cancer. The GWAS summary data was extracted from the Breast Cancer Association Consortium (BCAC) [including both estrogen receptor (ER)-negative and ER-positive breast cancer]; detailed information is shown in Supplementary table 3.

Statistical Analysis

In order to have an effective interpretation of MR analysis, the following three hypotheses must be satisfied[20]: (i) the IVs are strongly correlated with insomnia; (ii) the IVs influence lung cancer only through their effects on insomnia, and (iii) the IVs are independent of any confounders from the insomnia-lung cancer relationship.

The main and subgroup MR analyses were performed using the inverse-variance weighted (IVW) method. Generally, the IVW gives a consistent estimate of the causality; it consists of meta-analysis on each SNP's Wald ratio between the exposure and outcome using a random-effects inverse-variance method, which weights each Wald ratio based on its standard error (se) while considering possible measurable heterogeneity. In this regard, given the phenotype of insomnia (X) on the risk of lung cancer (Y) and the genetic variants (G) as IVs, the IVW analyses were conducted by combining genetic variants on insomnia (b_{YG}) with their standard errors (SE_{YG}) to examine the causality between genetically determined exposure factor (β_{MS}) and outcome phenotype ($\beta_{lung\ cancer}$). Odds ratios (ORs) with 95% confidence intervals (CIs) for lung cancer and both subtypes (i.e., lung adenocarcinoma and squamous cell lung cancer) were assessed and estimated. However, in the

presence of horizontal pleiotropy in invalid instrumental variables, the IVW estimate might be biased. In this case, the Mendelian Randomization Pleiotropy Residual Sum and Outlier (MR-PRESSO) test, the MR-Egger regression, and the weighted-median estimator were also performed to comprehensively evaluate the MR results[21]. The MR-PRESSO can detect horizontal pleiotropy and if necessary, it can also yield a corrected estimate by conducting outlier removal. The MR-Egger and the weighted-median estimator were more sensitive to horizontal pleiotropy though they were less statistically well-powered. The MR-Egger regression assumed the independence of the pleiotropic associations and conducted a weighted-linear regression of the outcome on the exposure[22]. The intercept of the MR-Egger regression offered an exploration of pleiotropy and the estimate of the causality adjusted for pleiotropy. Under the premise that more than 50% of the weights came from valid instrumental variables, the weighted median estimates could effectively pool the effects of individual variables[22].

Sensitivity Analyses

The leave-one-out analyses were performed to evaluate whether the results of the IVW method would be biased by one or several single sensitive SNPs or not. The MR-PRESSO global and distortion test[21] and the Egger intercept analysis (see Statistical Analyses) were performed to evaluate the horizontal pleiotropy. Additionally, the MR-heterogeneity test was also conducted to identify SNPs that are responsible for heterogeneity in casual estimation by performing Cochran's Q test on the IVW and the MR-Egger estimate[23].

The third assumption of the MR analysis indicates that the genetic variants contained in the IV set

are not allowed to associate with any other factors correlated with insomnia or lung cancer. Basic MR analyses would not be able to provide an accurate estimate of the causality of insomnia on lung cancer once our included insomnia-associated genetic variants were also linked to such confounders. Therefore, we attempted to explore whether the correlation between insomnia and lung cancer is driven by common risk factors for both insomnia and lung cancer.

First, smoking is one of the most well-known risk factors causing lung cancer. Alicia Nuñez et al.[24] reported strong evidence that smoking is associated with increased insomnia incidence and severity. Hence, smoking may be both a potential confounder and mediator of the insomnia-lung cancer correlation. In addition, cohort studies showed daily exercise may be associated with lung cancer and insomnia[25,26]. Second, alcohol consumption[27,28] was also proven to play a part in the incidence of insomnia and lung cancer as well. On top of smoking and alcohol, aging was also an important risk factor for insomnia, and all types of malignancies[29,30], thus, aging might be a confounder in the insomnia-lung cancer causality. Therefore, these three factors need to be considered as potential confounders of the insomnia-lung cancer relationship.

Based on the existing literature, we further sought to explain the potential mechanisms in the insomnia-lung cancer relationship as well as to investigate the suspected mediators. Basic medical studies[31,32] suggested that vitamin D may play an important role in the central nervous system (CNS), and there is increasing evidence[33,32] indicating that lower vitamin D levels are associated with an increased risk of mental disorder-induced insomnia. Meanwhile, vitamin D has long been considered to have important effects against cancer development[34], while current cohort studies[35,36] showed an inverse association between serum vitamin D and lung cancer risk.

Insomnia was reported to be strongly associated with cognitive dysfunction[37,38]. Studies focusing on the effects of insomnia on learning ability showed that insomnia patients may have difficulties in daily study activities[39,40]. The Mendelian Randomization study by Zhou et al. [41] indicated the causality between genetically predicted low levels of education and increased risk of lung cancer. In addition, pharmacological interventions including sedatives and hypnotics are often used on insomnia patients[42], but, evidence demonstrated that drug-induced mental disorders may be risk factors for malignancies[43-46]. As a result, it is vital to explore whether education, drug-induced mental disorders, and vitamin D intake are the mediators between insomnia and lung cancer risk.

Consequently, as smoking, daily exercise, aging, and alcohol consumption could be confounders, and smoking, vitamin D supplement, drug-induced mental disorders, and education could be regarded as mediators of the insomnia–lung cancer relation, we assessed the influence of smoking (Ever vs. never smoked, and previous and current smoking status), alcohol consumption, vitamin D supplement, daily exercise (frequency of exercises in last 4 weeks), aging (time to death), drug-induced mental disorders (mental and behavioral disorders due to sedatives or hypnotics), and education (year-ended, full-time education) with individual-level data in 6 subset studies from the GenBank, UK-Biobank, Pan-UK Biobank, Tobacco and Genetics consortium (TAG), Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE), and the Medical Research Council Integrative Epidemiology Unit (MRC-IEU) (Table 2). Information on suspected confounders and mediators of insomnia-lung cancer relation was collected in corresponding studies. All MR analyses were performed in R (version 4.0.4) using the package ‘TwoSampleMR’[47]

(version 0.5.6) and 'MRPRESSO' [21] (Version 1.0.0).

Ethical Statement

This study was approved by the ethics committee of The First Affiliated Hospital of Guangzhou Medical University. Considering that the study was a retrospective analysis, informed consent of all patients was waived by the ethics committee.

RESULTS

MR Estimates for Multi-Polymorphism Scores

Power calculation indicated that we had 80% power at a 0.05 significance level to detect an OR of 1.085 for lung cancer. The results of the MR analysis indicated an increased risk of lung cancer in insomnia patients (OR = 1.1671; 95% CI 1.0754–1.2666, $p = 0.0002$), lung adenocarcinoma (OR = 1.1878; 95% CI 1.0594–1.3317, $p = 0.0032$) and squamous cell lung cancer (OR = 1.1595; 95% CI 1.0248–1.3119, $p = 0.0188$) (Table 3). Subgroup analyses demonstrated null causal associations between morningness, sleep duration (average: 7.1 hours) ,and snoring, but it also demonstrated significant 1.51-fold and 1.94-fold increased risks between snapping and lung cancer and lung adenocarcinoma, respectively (Table 4). The MR results for breast cancer are shown in Supplementary table 3.

Assessment of MR assumptions

All chosen SNPs in the current study were selected at the genome-wide significance threshold of $p < 5\times10^{-8}$ to meet the first MR assumption. The leave-one-out analysis indicated no evidence that single SNP had impact on the overall effect of insomnia on lung cancer (Supplementary figure 1).

MR-PRESSO test and MR-Egger regression suggested no horizontal pleiotropy, which indicated no violation to the second assumption in subgroup analyses. Our MR-heterogeneity test showed the existence of heterogeneity in the correlation of overall lung cancer, but no heterogeneity was found in the adenocarcinoma and squamous cell carcinoma, suggesting the existence of histology-specificity of lung cancer. Results of the sensitivity analyses are listed in Supplementary table 4. As for the third MR assumption, we found no evidence that the included insomnia-associated SNPs were genome wide significantly associated with any other phenotype mentioned above as risk factors for both insomnia and lung cancer, suggesting no violation to this assumption. Furthermore, we evaluated if the correlation between genetically predicted insomnia and lung cancer was impacted by potential confounders (smoking, daily exercise, aging, and alcohol consumption) or mediators (smoking, vitamin D supplement, and education). The results of our analyses demonstrated that there was no statistical significance in the overall analyses between genetically predicted insomnia and potential confounders as well as mediators (Table 5). Therefore, this suggested that our genetic instrument of 148 insomnia-associated SNPs was not genome-wide significantly associated with any other phenotypes mentioned above as risk factors for both insomnia and lung cancer, which proved that the third MR assumption was not violated in this study.

DISCUSSION

Up until now, the relationship between lung cancer risk among patients with insomnia remains unknown due to the lack of epidemiological evidence. To our best knowledge, this is the first-ever MR analysis precisely investigating the causality between insomnia and lung cancer risk with less susceptibility to inverse causality and confounders, which may help us better understand and investigate if insomnia is a risk factor for lung cancer. Based on a total of 148 SNPs as IVs, our MR

analysis demonstrated that genetically predicted insomnia was causally associated with increased risks of overall lung cancer, lung adenocarcinoma, and squamous cell lung cancer.

Comparison with Previous Studies

Previous epidemiological evidence has revealed the potential risky effect of insomnia on site-specific malignancies. Fang et al. conducted a nationwide nested case-control study, demonstrating a 1.73-fold breast cancer risk[48]. Fleming et al. found that in breast cancer patients, the prevalence of insomnia was 8% before the diagnosis of breast cancer[49]. Sen et al. conducted the HUNT study in 2017 with 33,332 women and a mean of 14.7 years follow-up, and finally demonstrated that insomnia was twice as risky as to breast cancer in patients with insomnia suffering from the following symptoms: difficulty initiating sleep, difficulty maintaining sleep, and having non-restorative sleep[50]. Finally, a recent retrospective case-control study revealed an elevated 1.54-fold colorectal cancer risk among 7,355 patients with insomnia[51]. These studies indicated that site-specific cancer risks may elevate in patients with insomnia. Nevertheless, up till now, information on patients with insomnia regarding the risk of lung cancer is scanty. A Korean study demonstrated an increased risk of lung cancer among patients with insomnia who are under sedative-hypnotic drugs as compared to non-drug users, however, it did not show the specific lung cancer risk of patients with insomnia without medication use compared with the general population[46]. Recently, Xie et al. [11] conducted a prospective cohort study in UK Biobank and evaluated the relationships between sleep traits and lung cancer risk. This study provided the first-ever observational evidence for insomnia-lung cancer relationship, though it showed a null association between insomnia and lung cancer risk, which is contrary to our MR results. However,

this study indicated that due to the relatively long induction time of lung cancer onset or diagnosis, the characteristics of sleep traits at recruitment might not reflect the appropriate etiological window of lung cancer. Meanwhile, although this study included the potential confounding factors like smoking, alcohol consumption and sedative use into the multivariable model, unmeasured and residual bias remained unassessed.

Moreover, due to the flaws of observational study design, reverse causation may bias the results. At the same time, such conflicting findings are likely to be related to a variety of potentially confounding factors including duration of the studies, differences in cancer screening programs across different countries, methods of data collection, population-specific genetics, and environmental exposures. Current epidemiological evidence has not evaluated the influence of potential confounding factors including smoking status, alcohol consumption, exercise frequency, and so on, which may bias the true association between insomnia and lung cancer. Thus, it is not enough to draw a definite conclusion on the causality between insomnia and lung cancer. The result of the current MR study demonstrated that genetically predicted insomnia indeed has a causal association with an increased lung cancer risk, which could be a reference for the blank in this field and could inspire the initiation of a number of high-standard, large-scale, and long-time cohort studies to explore the potential relationship between insomnia and lung cancer.

Potential Mechanisms for the Observed Associations

In the past, lung cancer has widely been considered a proxy for smoking. Though the existing observational studies have been progressively charting a spectrum of lung cancer risk factors,

researchers cannot completely exclude the potential impact of smoking from these latent risk factors. In view of this, the MR analysis provided us the opportunity to screen the risk factor for lung cancer, while avoiding the influence of reverse causality and confounding factors to the greatest extent. Alicia Nuñez et al.[24] reported strong evidence that smoking is associated with increased insomnia incidence and severity. Chen et al. [52] also demonstrated a 1.78-fold increased risk for clinically diagnosed insomnia. Hence, smoking may be the risk factor for not only lung cancer but also insomnia, indicating that smoking may be both a potential confounder and mediator of the insomnia-lung cancer association. In fact, it is difficult to determine directly whether the SNPs associated with insomnia would be affected by smoking. According to the analysis method by Zhou et al. [41], we noticed that it is possible to indirectly detect whether smoking affects the insomnia-lung cancer association. To do this, we performed MR analyses again to explore whether SNPs-insomnia and SNPs-smoking are related, and the results showed that the p values were both >0.05 (Table 5), which indirectly excludes the influence of smoking on the insomnia-lung cancer association.

Several plausible biological models have been put forward to explain the observed elevated cancer risk. Modern studies have proposed other suggestions of clock-gene deregulation-induced tumor genesis and progression[53], sleep deprivation-induced immunosuppression[54], and the insomnia-induced altered melatonin secretion patterns[55-57] (i.e., timing, amount, and secretion duration); melatonin has been found to have multiple anti-tumor effects such as modulation of the cell cycle and apoptosis, stimulation of cell differentiation, anti-oxidant effect, and the prevention of chronodisruption (CD), which was found to have frequent occurrences among lung cancer patients[58-60]. Insomnia can lead to CD, which can subsequently result in endocrinological,

physiological, and sleep-wake cycle alterations, and this may result in an increased lung cancer risk[61].

Our results suggested a statistically significant increased risk (OR = 1.19, p = 0.0031) in lung adenocarcinoma, but a not that significant association (OR = 1.16, p = 0.0188) between insomnia and squamous cell lung carcinoma. The underlying mechanisms explaining the intriguing histology-specific difference included the insomnia-induced immunosuppression, and the differential expression of estrogen receptors (ERs) in lung adenocarcinoma and squamous cell lung carcinoma; estrogen and ERs are known to play an important role in the carcinogenesis of non-small cell carcinoma (NSCLC) [62,63]. The most common models for immunosuppression are organ transplantation and human immunodeficiency virus (HIV) infection. Comprehensive analyses well interpreted the spectrum of cancer risks among solid organ transplant recipients and HIV-infected individuals, indicating the existence of site-specificity in cancer risks in immunosuppressive models[64-66]. However, Triplette et al.[67] demonstrated that the post-lung transplantation incidence of squamous cell lung carcinoma was higher than that of lung adenocarcinoma; this result was consistent in a cohort study focusing on lung cancer incidence among HIV-infected individuals[68], indicating that this histology-specific difference might not be well explained by insomnia-induced immunosuppressive status. Another potential mechanism is the differentiated expression of ERs. Previous studies have well demonstrated the role of estrogen and ER in the carcinogenesis of NSCLC[62,63] and the difference of expression in different subtypes of NSCLC; the ER expression is more abundant in adenocarcinomas than in squamous cell carcinomas[69,70]. Insomnia could induce the suppression of melatonin secretion, which in turn leads to an increase in

estrogen secretion levels, leading to the increased risk of adenocarcinoma rather than squamous cell carcinoma[71,72]. This is one of the possible explanations for this difference in our analysis. Future studies are needed to unravel this mystery.

Strengths and Limitations

There are a number of strengths in our study. First, it is the first-ever MR analysis to assess the causal relationship between insomnia and lung cancer. As it's shown above, our study design considered the effects of reverse causality and potential confounders. Second, our included 148 insomnia-associated SNPs were obtained from the published GWASs of the European ancestry, which are more convincing and can better explain the variation of insomnia. Third, our genetic summary data of lung cancer were extracted from ILCCO, an authoritative lung cancer consortium comprising 29,266 cases and 56,450 controls of the European ancestry. With a large sample size and robustly associated IVs, this MR analysis could provide us with adequate statistical power and a relatively accurate estimation of causal effects. In addition, investigations of potential confounders and mediators were conducted to explore whether the correlation between genetically predicted insomnia and lung cancer would be affected by them.

Despite the aforementioned strengths of our study, we cannot ignore the limitations. Firstly, with approximately 17% higher risks in both overall lung cancer and lung adenocarcinoma, the observed causal effect between insomnia and lung cancer was modest, which consequently indicated the relatively limited significance of insomnia in the disease development of lung cancer. Future GWASs with strong IVs are needed to explain more heritability of insomnia, which could provide us with more data to work with in our MR analysis to determine a more accurate causal effect

between insomnia and lung cancer, which in turn can provide more reliable guidance for public health strategies/interventions. Second, we found that there was heterogeneity in lung cancer overall. Thus, stratified analyses based on lung cancer subtypes were performed to explore the potential heterogeneity. The results of the stratified analyses suggested no heterogeneity in lung adenocarcinoma and squamous cell lung carcinoma, indicating that the histology-specificity might be the origin of the heterogeneity in overall lung cancer. Besides, in confounding factors analyses, the number of SNPs of aging (time to death) was insufficient, which might affect the accuracy and persuasiveness in confounding factor analysis of aging. Future GWASs would provide more aging-related SNPs, on the basis of which we could better explore the role of aging in the insomnia-lung cancer relationship. Another limitation is that all participants were of European descent, this lack of data in other regions and populations could also influence the results of our study since it limits the applicability. In addition, considering that some SNPs we used in the MR analysis may be related to some unknown factors relating to the risk of lung cancer, we cannot completely exclude the possible impact of the horizontal pleiotropic effect on the results.

Due to the lack of a strict diagnostic criteria of insomnia, which is often diagnosed based on multiple assessments (consultation, subjective statement, and objective assessment), we thought that any form of insomnia diagnosis may be biased. In the Jansen et al. study, insomnia cases were defined as participants who answered with "usually" to the question of "Do you have trouble falling asleep at night or do you wake up in the middle of the night?". The differences in diagnostic criteria of insomnia would make it difficult to produce consistent outcomes. The common problem of GWAS research is the limitation between phenotype and sample; if the phenotype was strictly controlled,

researchers would not get enough samples to analyze the results comprehensively and stably, on the other hand, if the phenotype was not strictly controlled, it would lead to some deviation to the results, leading to a weaker outcome. It's inevitable in the GWAS of insomnia from the Jansen et al. study, but it can be said to be the optimal solution under the existing conditions. Future GWAS research needs to be conducted with more consistent diagnostic criteria for insomnia, as well as a large sample size.

CONCLUSION

In conclusion, our MR analysis firstly indicated that genetically predicted insomnia was causally related to an increased lung cancer risk among people of European ancestry, which suggested the causality of insomnia in the development of lung cancer. However, due to the limitations of available data, some vital impactors were not able to be taken into account, indicating that we could not rule out the possible impact of pleiotropy completely. In addition, due to the lack of evidence both on the epidemiology and mechanism level, better designed studies are needed to further interpret the results in our study.

Disclosures

All the authors declare no conflicts of interest.

Contributors

All authors contributed to the design of the study and to the drafting of the paper and have seen and approved the final version.

Acknowledgements

The authors acknowledge the efforts of the consortia in providing high-quality GWAS resources for researchers. Data and material are available from corresponding GWAS consortium.

The authors thank Shengye Niu (HBSc, University of Toronto) for helping edit the manuscript.

Funding

This study was supported by the National Natural Science Foundation of China (Grant No.81871893) and Key Project of Guangzhou Scientific Research Project (Grant No.201804020030) provide and maintain the database.

Supplemental Materials

Supplementary figure 1. Leave-one-out of SNPs associated with insomnia and lung cancer.

Supplementary table 1. Detailed information of initial 250 insomnia-related SNPs and 447 SNPs of other sleep traits.

Supplementary table 2. Detailed information of selected 148 SNPs.

Supplementary table 3. Detailed information and association between insomnia and the risk of breast cancer and its subtypes.

Supplementary table 4. Sensitivity Analysis Results for MR Analyses of Insomnia to Lung Cancer.

Supplementary table 5. Association between each SNP related to insomnia and the risk of lung cancer and its subtypes.

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Table 1. Details of studies included in Mendelian randomization analyses.

Trait	Consortium	Number of cases	Number of controls	Sample size	Population	PubMed ID	Year
Lung cancer	ILCCO	29,266	56,450	85,716	European	28604730	2017
Lung adenocarcinoma	ILCCO	11,273	55,483	18,336	European	28604730	2017
Squamous cell lung cancer	ILCCO	7,426	55,627	63,053	European	28604730	2017

ILCCO, International Lung Cancer Consortium

Table 2. Details of studies of confounders and mediators.

Trait	First author	Consortium	Study participants	Year	PubMed ID	Website
Ever vs never smoked	Furberg	TAG	74,035	2010	20418890	www.med.unc.edu/pgc/results-and-downloads
Former vs current smoker	Furberg	TAG	74,035	2010	20418890	www.med.unc.edu/pgc/results-and-downloads
Alcohol consumption	Clarke	UK Biobank	112,117	2017	28937693	https://www.ukbiobank.ac.uk/
Aging (time to death)	Stefan Walter	CHARGE	25,007	2011	21782286	https://www.chargeconsortium.com/
Vitamin D	Ben Elsworth	MRC-IEU	460,351	2018	NA	https://www.bristol.ac.uk/integrative-epidemiology/
Frequency of other exercises in last 4 weeks	Pan-UKB team	Pan-UK Biobank	1,112	2020	NA	https://pan.ukbb.broadinstitute.org/
Year ended full time education	Ben Elsworth	MRC-IEU	112,569	2018	NA	https://www.bristol.ac.uk/integrative-epidemiology/
Mental and behavioral disorders due to sedatives or hypnotics	NA	GenBank	94,964	2020	NA	https://www.ncbi.nlm.nih.gov/genbank

NA, not available; TAG, Tobacco and Genetics consortium; Pan-UKB, Pan-UK Biobank; MRC-IEU, Medical Research Council Integrative Epidemiology Unit; CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology

Table 3. Mendelian randomization estimates of the associations between insomnia and risk of lung cancer.

Outcome	IVW method		MR-PRESSO		Weighted median method		MR-Egger	
	OR (95% CI)	*p value*	OR (95% CI)	*p value*	OR (95% CI)	*p value*	OR (95% CI)	*p value*
Lung cancer	1.1671 (1.0753, 1.2666)	0.0002	1.1582 (1.0704, 1.2532)	0.0003	1.1382 (1.0195, 1.2707)	0.0166	1.1698 (0.8500, 1.6097)	0.3372
Lung adenocarcinoma	1.1878 (1.0594, 1.3317)	0.0031	1.1720 (1.0431, 1.3167)	0.0084	1.1192 (0.9402, 1.3325)	0.2052	1.4892 (0.9592, 2.3401)	0.0777
Squamous cell carcinoma	1.1595 (1.0248, 1.3119)	0.0188	1.1648 (1.0339, 1.3123)	0.0131	1.0784 (0.8980, 1.2952)	0.4188	0.7914 (0.4947, 1.2660)	0.3307

IVW, inverse-variance weighted; MR, mendelian randomization; OR, odds ratio; CI, confidence interval; MR-PRESSO, Mendelian Randomization Pleiotropy Residual Sum and Outlier

Table 4. IVW estimates of the causal associations between other sleep traits and risk of lung cancer.

Outcome	Sleep Duration (Mean: 7.1h)			Morningness			Napping			Snoring		
	nSNP	OR (95% CI)	*p value*	nSNP	OR (95% CI)	*p value*	nSNP	OR (95% CI)	*p value*	nSNP	OR (95% CI)	*p value*
Lung cancer	42	0.7812 (0.5110, 1.2034)	0.2658	136	1.0585 (0.8532, 1.3157)	0.6008	6	1.5140 (1.1675, 1.9634)	0.0017	31	0.9768 (0.7469, 1.2775)	0.8641
Lung adenocarcinoma	42	0.9133 (0.5438, 1.5338)	0.7317	136	1.1943 (0.8771, 1.6262)	0.2596	6	1.9404 (1.3006, 2.8948)	0.0011	31	0.9120 (0.6608, 1.2587)	0.5752
Squamous cell carcinoma	42	1.1891 (0.6143, 2.2787)	0.615	136	1.0644 (0.7695, 1.4723)	0.7059	6	1.2333 (0.8296, 1.8334)	0.2999	31	0.8785 (0.5435, 1.4196)	0.5965

IVW, inverse-variance weighted method; OR, odds ratio; CI, confidence interval; nSNP, number of single nucleotide polymorphism

Table 5. Causal effects between genetically predicted insomnia and confounders and mediators.

Outcomes	nSNP	Causal effect (95% CI)	*p* value
Ever vs never smoked	117	1.0345 (0.9490, 1.1277)	0.4401
Former vs current smoker	117	0.9368 (0.8547, 1.0268)	0.1632
Alcohol consumption	148	0.9941 (0.9810, 1.0074)	0.3904
Vitamin D	148	1.0728 (0.9161, 1.2564)	0.3828
Frequency of other exercises in last 4 weeks	146	1.0722 (0.9184, 1.2519)	0.3771
Year ended full time education	150	0.9835 (0.9615, 1.0060)	0.1502
Mental and behavioral disorders due to sedatives or hypnotics	116	0.9773 (0.7228, 1.3213)	0.8814
Aging (time to death) (unit decrease)	2	1.0094 (0.9764, 1.0435)	0.1438
Aging (time to death) (unit increase)	1	1.0344 (0.9885, 1.0825)	0.5810

CI, Confidence interval; nSNP, number of single nucleotide polymorphism

Highlights

1. Few previous studies have explored the relationship between insomnia and lung cancer.
2. Mendelian Randomization made us able to evaluate the insomnia-lung cancer causality.
3. Genetically-predicted insomnia was causally related to an increased lung cancer risk.
4. Future studies are needed to better elucidate the insomnia-lung cancer relationship.

CRediT Author Statement

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