Gene–environment interaction effect of HPA axis gene polymorphisms and job stress on the risk of sleep disturbances (#91147)

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Gene–environment interaction effect of HPA axis gene polymorphisms and job stress on the risk of sleep disturbances

Min Zhao [1], **Yuxi Wang** [1], **Yidan Zeng** [1], **Huimin Huang** [1], **Tong Xu** [2], **Baoying Liu** [1], **Chuancheng Wu** [1], **Xiufeng Luo** [3], **Yu Jiang** [Corresp. 1]

[1] Fujian medical university, Fuzhou, China

[2] Affiliated Zhongshan Hospital of Dalian University, Dalian, China

[3] Fuzhou Municipal Center for Disease Control and Prevention, Fuzhou, China

Corresponding Author: Yu Jiang
Email address: jiangyu@fjmu.edu.cn

Background. Studies have shown that long-term exposure to job stress may increase the risk of sleep disturbances and that hypothalamic–pituitary–adrenal (HPA) axis gene polymorphisms may play an important role in the psychopathological mechanism underlying sleep disturbances. However, the interaction among job stress, gene polymorphisms and sleep disturbances have not been examined from the perspective of the HPA axis. This study aimed to know whether job stress is a risk factor for sleep disturbances and further explore the effect of the HPA axis genes × job stress interaction on sleep disturbances among railway workers.

Methods. In this cross-sectional study, 671 participants (363 males and 308 females) from the China Railway Fuzhou Branch were included. Sleep disturbances were evaluated with the Pittsburgh Sleep Quality Index (PSQI), and job stress was measured with the Effort-Reward Imbalance scale (ERI). Generalized multivariate dimensionality reduction (GMDR) models were used to assess gene-environment interactions.

Results. We found a significant positive correlation between job stress and sleep disturbances ($P<0.01$). The FKBP5 rs1360780-T and rs4713916-A alleles and CRHR1 rs110402-G allele were associated with increased sleep disturbances risk, with adjusted ORs (95% CI) of 1.75 (1.38-2.22), 1.68 (1.30-2.18) and 1.43 (1.09-1.87), respectively. However, the FKBP5 rs9470080-T allele was a protective factor against sleep disturbances, with an OR (95% CI) of 0.65 (0.51-0.83). GMDR analysis indicated that under job stress, individuals with the FKBP5 rs1368780-CT, rs4713916-GG, rs9470080-CT genotypes and the CRHR1 rs110402-AA genotype had the highest sleep disturbances risk.

Conclusions. Individuals carrying the risk alleles who experience job stress may be at increased risk of sleep disturbances. These findings may be used to improve sleep disturbances in the future.

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Min Zhao[1#], Yuxi Wang[1#], Yidan Zeng[1], Huimin Huang[1], Tong Xu[3], Baoying Liu[1], Chuancheng Wu[1], Xiufeng Luo[2], Yu Jiang[1]

[1] Department of Preventive Medicine, Fujian Provincial Key Laboratory of Environment Factors and Cancer, Key Laboratory of Environment and Health, School of Public Health, Fujian Medical University, Fuzhou, China
[2] Department of Occupational Health, Fuzhou Municipal Center for Disease Control and Prevention, Fuzhou, China
[3] PET/CT Center, Key Laboratory of Functional Molecular Imaging, Affiliated Zhongshan Hospital of Dalian University, Dalian 116001, China
[#] These authors contributed equally to this work.

Corresponding Author:
Yu Jiang[1]
Department of Preventive Medicine, School of Public Health, Fujian Medical University, Fuzhou, 350122, China
Email address: jiangyu@fjmu.edu.cn

Abstract

Background. Studies have shown that long-term exposure to job stress may increase the risk of sleep disturbances and that hypothalamic–pituitary–adrenal (HPA) axis gene polymorphisms may play an important role in the psychopathological mechanism underlying sleep disturbances. However, the interaction among job stress, gene polymorphisms and sleep disturbances have not been examined from the perspective of the HPA axis. This study aimed to know whether job stress is a risk factor for sleep disturbances and further explore the effect of the HPA axis genes × job stress interaction on sleep disturbances among railway workers.

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Conclusions. Individuals carrying the risk alleles who experience job stress may be at increased risk of sleep disturbances. These findings may be used to improve sleep disturbances in the future.

Introduction

Sleep is essential for humans, helping to maintain energy, promote growth and development, and improve immunity (Ramar et al., 2021). However, ~~with lifestyle changes~~, sleep disturbances have seriously reduced people's quality of life and have become a major public health problem that impacts people's physical and mental health (Halonen et al., 2017). The global prevalence of sleep disturbances is approximately 37.9% (Wu et al., 2021); Canada has a prevalence of 23.8% (Chaput et al., 2018) , Japan of 13.3% (Miyachi et al., 2021) , and China of 29.2% (Shi et al., 2020); this prevalence rate is increasing. Studies have shown that long-term sleep disturbances damage people's physical and mental health and are an early risk factor for many diseases, such as cardiovascular and cerebrovascular diseases, neuropsychiatric disorders, accidental injuries and even death (Rajaratnam et al., 2011;Morin and Jarrin 2022).

Job stress refers to negative physical and psychological reactions that occur when job requirements do not match workers' abilities, coping resources and demands (Basu et al., 2017). In recent years, numerous studies have shown that excessive job stress can lead to imbalances in physiological functions, resulting in decreased sleep quality at night and sleep problems such as insomnia and drowsiness (Khamisa et al., 2016;Herr et al., 2018;D'Ettorre et al., 2020). Therefore, job-related stress is a major occupational risk factor that significantly increases the risk of sleep disturbances (Juster and McEwen 2015;Linton et al., 2015). Epidemiological research has indicated that job stress is related to an increased risk of sleep disturbances (Blom et al., 2020;Hämmig 2020). A cohort study of workers in Denmark aligns with this conclusion (Nordentoft et al., 2020). In addition, sleep disturbances also seriously affect the efficiency of workers, leading to a decline in production efficiency and the occurrence of accidents, resulting in substantial social and economic burdens (Kucharczyk et al., 2012;Uehli et al., 2014). It is essential to explore the mechanism underlying the influence of job stress on sleep disturbances among occupational groups and to take active measures to reduce the occurrence of sleep disturbances.

The hypothalamic–pituitary–adrenal (HPA) axis is thought to be the main pathway mediating the stress response (Hirotsu et al., 2015). More importantly, the HPA axis regulates the sleep-wake cycle: activation of the HPA axis may lead to awakening and insomnia in animals and humans (de Feijter et al., 2022). Dysfunction of the HPA axis in any component (of the

corticotropin-releasing hormone receptor, glucocorticoid receptor or mineralocorticoid receptor) may disturb sleep (Buckley and Schatzberg 2005). When encountering stressors (physiological or psychological), the hypothalamus releases corticotropin-releasing hormone (CRH). CRH stimulates the anterior pituitary to release corticotropin, and corticotropin activates the adrenal cortex to upregulate the production of glucocorticoids (GCs). Its main function is to restore internal physiological balance after exposure to stress. However, Weitzman et al. (Weitzman et al., 1983) showed that the release of GCs was related to the occurrence and development of sleep disturbances. Moreover, most stress-related hormones promote wakefulness, and elevated HPA activity appears to contribute to stress-induced insomnia (Nicolaides et al., 2000). Exploring the genes that play a role in HPA axis regulation may be useful in determining the relationship between job stress and sleep disturbances. Gerritsen et al. suggested that the CRH gene is linked to stress and sleep disturbances (Gerritsen et al., 2017). In addition, individual variation in the FK506 binding protein 5 (FKBP5) gene is related to an imbalance in the HPA axis; this imbalance has been identified as the key neurobiological mechanism underlying psychotic symptoms. An animal study also reported that FKBP5 may be a target gene for stress-induced mood and sleep disturbances (Albu et al., 2014). Although many studies have shown that sleep disturbances are related to HPA axis genes and job stress, their interaction and effect on sleep disturbances are still unclear.

In recent years, many researchers have assessed the effects of gene–environment interactions on sleep disturbances (Zwicker et al., 2018;Zhang et al., 2022). Both genetic (Federenko et al., 2004) and environmental factors have been shown to influence an individual's cortisol response to stress through the HPA axis, even response extreme enough to increase the risk of sleep disturbances (Foley and Kirschbaum 2010;Kudielka and Wüst 2010). Moreover, interactions between some genes (the glucocorticoid receptor [GR] (Bakker et al., 2017), FKBP5 (Matosin et al., 2018;Normann and Buttenschøn 2020), 5-hydroxytryptamine transporter [5-HTTLPR] (Huang et al., 2014) and dopamine D2 receptor [DRD2] (Jiang et al., 2020) and exposure to job stress have repeatedly been found to play a role in the onset of sleep disturbances. For instance, Brummett et al. (Brummett et al., 2007) found that the 5-HTTLPR gene polymorphism is related to sleep quality problems in individuals exposed to long-term stress. A previous study reported that the effects of early life stress on mental illnesses such as sleep disturbances are more prominent in the G allele of the GR gene rs258747 and rs41423247 (Lian et al., 2014). One of the largest Trier Social Stress Test (TSST) cohorts indicated that the interactions among FKBP5, corticotrophin-releasing hormone receptor type 1 gene (CRHR1) gene polymorphisms and psychosocial stress may affect the cortisol response and cause circadian rhythm disorders (Mahon et al., 2013). However, there are still SNPs in the HPA axis that have not been fully investigated in these interactions. More importantly, most studies have limited their focus to the effect of a single gene-stress interaction on sleep quality, and few have examined multiple major genes regulating the HPA axis to determine the relationships among gene polymorphisms, job stress, and their interaction with sleep disturbances.

Therefore, we examined the independent and interactive effects of HPA axis gene polymorphisms and job stress on sleep quality among front-line railway workers in Fuzhou City, China. Our investigation focused on the interaction effect of genetic and environmental factors on sleep disturbances to provide new insights for improving sleep health.

Materials & Methods

Subjects

The present study was conducted as part of an Occupational Health Study for Railway Workers (OHSRW) between October 2019 and May 2020. Inclusion and exclusion criteria have been described in detail in a previous article (Wang et al., 2022). A set of self-report questionnaires was used to collect information on demographic characteristics, sleep disturbances and job stress. As a part of physical examinations, 5-mL fasting venous blood samples were collected from each subject at the workplace between 7:00 am and 9:00 am. In this cross-sectional study, a total of 690 participants were enrolled, of whom 19 were excluded due to insufficient information or missing blood samples. Ultimately, 671 (males/females =363/308) railway front-line workers were included in the final analysis. This study was approved by the Ethics Committee of Fujian Medical University (No.2019025). All subjects provided informed consent before they participated in the study.

Job stress

The Effort-Reward Imbalance (ERI) scale was used to evaluate job stress, which is based on Siegrist's ERI model (Siegrist and Li 2017). Cronbach's alpha of this scale was 0.882. The ERI questionnaire includes a total of 23 items in three dimensions: Job effort (6 items), Job reward (6 items) and Overcommitment (11 items). Each of the items is evaluated on a 5-point scale (from 1 to 5). The ERI score evaluation method is as follows: each item is assigned the same weight, and the ERI score is calculated as E/(R × (6/11)). ERI scores>1 indicate an imbalance between effort and reward, which is considered to reflect job stress (Choi et al., 2014).

Sleep disturbances

The Pittsburgh Sleep Quality Index (PSQI) was used to assess the sleep quality of the subjects (Buysse et al., 1989). The PSQI has shown strong reliability and validity in a variety of samples, indicating that this questionnaire provides a good understanding of sleep disturbances (Mollayeva et al., 2016). The PSQI consists of 7 components: subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbance, sleep medication, and daytime dysfunction. Each dimension is graded on a score ranging from 0 to 3, and the total PSQI score ranges from 0 to 21. In this study, subjects with a global score higher than 5 were classified as experiencing sleep disturbance (Liu et al., 2021).

DNA Extraction and Genotyping

After a 12-hour fast, venous blood samples were collected from all participants using EDTA-containing tubes. Genomic DNA was isolated and purified from the samples using a whole blood genome extraction kit (Beijing Thinkout Sci-Tech Co., Ltd), and the extracted DNA was stored in a -80°C freezer. Gene polymorphisms were detected by the SNaPshot method (Larsson et al., 2022). Tag single nucleotide polymorphisms were derived from a Chinese Han population in the Haplotype Map database (National Center for Biotechnology Information) (Sayers et al., 2023). We explored polymorphisms of several major genes that regulate the HPA axis: the FKBP5 gene (rs1360780, rs3800373, rs9470080, rs4713916, rs3777747, and rs9296158), CRHR1 (rs110402), corticotrophin-releasing hormone type 2 receptor gene (CRHR2; rs2267715), and the glucocorticoid receptor gene (NR3C1; rs41423247). Table 1 shows the sequences of the primers.

Confounding Factors

It has been demonstrated that some demographic, socioeconomic and lifestyle factors are related to sleep disturbances; thus, they may influence the results of any interaction between sleep disturbances and job stress or HPA axis gene polymorphisms (Wakasugi et al., 2014). The variables we included as confounders have been described in previous articles (Wang, Zhao et al., 2022). In particular, smoking and drinking alcohol were considered potential confounding lifestyle factors.

Statistical Analysis

Statistical analyses were carried out using SPSS version 26.0 (SPSS Inc., Chicago, IL, USA). ERI and PSQI scores are presented as the mean ± standard deviation (SD). Demographic data between two groups were compared using the chi-squared test for categorical variables. The Hardy-Weinberg equilibrium (HWE) for the HPA axis gene polymorphisms was tested using a chi-squared goodness-of-fit test. Pearson correlation analysis was used to assess the correlations of job stress with sleep disturbances and its dimension scores. After adjusting for sex, age, ethnicity, marital status, smoking status and drinking status as covariates, odds ratios (ORs) and 95% confidence intervals (Levante et al.,) were determined for the association of genotypes and job stress with the risk of sleep disturbances by logistic regression. Bonferroni correction was applied to account for multiple comparisons. Furthermore, GMDR (http// sourceforge.ne/projects/gmdr/) was used to identify the best HPA axis gene × job stress combination (Xu et al., 2016). We conducted a 10-fold cross-validation (CV) to avoid unstable results and obtained a robust averaged result. We also conducted locus and haplotype analysis for haplotypes associated with sleep disturbances using SHEsis (http://analysis.bio-x.cn). All reported *P* values are two-tailed, and those less than 0.05 were considered statistically significant.

Results

Demographic characteristics of the subjects

The general demographic characteristics of the sleep-disturbance group and nonsleep-disturbance group are summarized in Table 2. A total of 671 subjects were included in this study, including 269 with sleep disturbances and 402 without sleep disturbances. The incidence of sleep disturbances was 40.09%. We found no significant differences in sex, age, ethnicity, marital status, smoking status or drinking status between the two groups (P>0.05). In addition, there was a significant difference in the distribution of job stress between the two groups (P<0.01).

Correlation between job stress and sleep disturbances

Table 3 shows the correlations among the ERI scores, PSQI scores, and all dimensions of sleep disturbances. When sex, age, ethnicity, marital status, smoking status, and drinking status were controlled as covariates, the ERI score was positively correlated with three dimensions of sleep disturbances, including subjective sleep quality and sleep latency (P<0.01). Specifically, there was a positive correlation between ERI and PSQI scores, indicating that job stress is related to sleep disturbance, and the greater the job stress was, the higher the risk of sleep disturbances.

Associations of 9 HPA axis SNPs with sleep disturbances

The associations of 9 SNPs in the HPA axis with sleep disturbances are presented in Table 4. We found that the FKBP5 rs1360780-TT genotype was associated with increased sleep disturbance risk, with an adjusted OR (95% CI) of 5.34 (3.02-9.44) (P=0.001, Bonferroni-corrected P<0.01). In contrast, the FKBP5 rs9470080-TT genotype was a protective factor against sleep disturbances, with an adjusted OR (95% CI) of 0.51 (0.28-0.92) (P=0.001, Bonferroni-corrected P<0.01). Regarding alleles, the FKBP5 rs1360780-T and rs4713916-A alleles and CRHR1 rs110402-G allele were risk factors for sleep disturbances, with adjusted ORs (95% CI) of 1.75 (1.38-2.22), 1.68 (1.30-2.18) and 1.43 (1.09-1.87), respectively (all P=0.001, Bonferroni-corrected P<0.01). However, the FKBP5 rs9470080-T allele was a protective factor against sleep disturbances, with an OR (95% CI) of 0.65 (0.51-0.83) (P=0.001, Bonferroni-corrected P<0.01). Haplotype analysis results showed that there were significant differences in haplotypes between the sleep-disturbance group and the nonsleep-disturbance group. The C-A-G-A-G-C haplotype was associated with an increased risk of sleep disturbance, and details are provided in the supplemental file (Table S1).

Effect of the gene–environment interaction on sleep disturbance

The best gene–environment interaction models were determined by GMDR analysis (Table 5). These models showed a significant effect of the interaction between HPA axis genes, job stress on sleep disturbance. The model had the maximum cross-validation consistency coefficient (10/10), and the accuracy of the training set and testing set was 0.68 and 0.60, respectively. This suggests that the best interaction model was the interaction between job stress and FKBP5 rs1360780, rs9470080, and rs4713916 genotypes and the CRHR1 rs110402 genotype. Furthermore, after controlling for the covariates, we also found that under job stress, the subjects

with the FKBP5 rs1368780-CT, rs4713916-GG, and rs9470080-CT genotypes and the CRHR1 rs110402-AA genotype had the highest sleep disturbance risk (Figure 1).

Discussion

To our knowledge, this is the first study to investigate the association among multiple HPA axis gene polymorphisms, job stress, and their interaction with sleep disturbances. Our study has three main findings as follows. (a) After controlling for confounding factors such as sex, age, and ethnicity, job stress was correlated with sleep disturbances. (b) The FKBP5 rs1360780-T and rs4713916-A alleles and the CRHR1 rs110402-G allele were associated with the risk of sleep disturbances. In contrast, the FKBP5 rs9470080-T allele was a protective factor against sleep disturbances. (c) GMDR analysis showed that in individuals under job stress, the risk of sleep disturbances was the highest with the FKBP5 rs1368780-CT, rs4713916-GG, and rs9470080-CT genotypes and the CRHR1rs110402-AA genotype.

In this study, we found that ERI scores were positively correlated with PSQI scores. The results showed that the higher the job stress experienced, the worse the sleep quality. Consistent with previous studies, a meta-analysis showed that high job stress was associated with a greater risk of insomnia (Yang et al., 2018). Lallukka et al. reached the same conclusion (Lallukka et al., 2014). Job stress is a very influential environmental factor for sleep (Gosling et al., 2014). There is evidence that the cortisol level increases in individuals experiencing job stress, and the HPA axis of people experiencing job stress may release cortisol that causes sleep disturbances (Rotvig et al., 2019). In addition, Birch (Birch and Vanderheyden 2022) explored thatr job stress mediates stress-induced insomnia by regulating the glucocorticoid signaling pathway in brain glial cells. This evidence suggests that job stress interferes with normal sleep and even increases the risk of sleep disturbances by activating the HPA axis.

Consistent with previous results, our study also revealed correlations between several major HPA axis regulatory genes and sleep disturbances. This result indicates that individuals with the FKBP5 rs1360780-T and rs4713916-A allele and the CRHR1 rs110402-G allele had a higher sleep disturbance risk. This is in line with a study by White et al. (White et al., 2012) that showed that the interaction between FKBP5 minor allele carriers and emotional neglect may increase the risk of stress-related disorders such as sleep disturbances. In addition, previous studies have shown that participants with the CRHR1 rs110402-A allele had higher cortisol levels 15 minutes post-stress, implying a risk of sleep disturbances in the future (Weeger et al., 2020). A meta-analysis showed that individuals exposed to stress and carrying the rs1360780-T allele or rs3800373-C allele had significantly shorter sleep durations and higher risks of stress-related diseases (Wang et al., 2018). Moreover, a study by Maguire et al. (Maguire et al., 2020) suggested that stress-related alterations of the HPA axis genes in PTSD may contribute to sleep difficulties. We also found a protective effect of the FKBP5 rs9470080-TT genotype against sleep disturbances, which contradicts the results of another study (Li et al., 2019). A possible explanation might be differences in the questionnaires and evaluation criteria.

Our findings provide new insights into the effects of gene-environment interactions on sleep disturbances. We found that the HPA axis gene × job stress interaction greatly affects sleep disturbances. More importantly, the GMDR results showed that individuals with the FKBP5 rs1360780-CC genotype, rs9470080-CC genotype and CRHR1 rs110402-AA genotype have the highest risk of sleep disturbances under job stress. Previous studies have also found effects of gene–environment interactions on sleep disturbances. For example, Zimmermann et al. (Zimmermann et al., 2011) found that individuals possessing risk alleles of two FKBP5 SNPs (rs3000377 and rs47139611) have the highest risk of reduced sleep quality if they have experienced adverse life events. Similar results were found in the interaction between childhood trauma and risk alleles of these SNPs (Bevilacqua et al., 2012). Likewise, He et al. (He et al., 2019) investigated 712 participants in a large general hospital in Beijing, and the results suggested that when experiencing work-related stress, individuals with the CRHR1 rs110402-A allele may experience reduced sleep quality. In summary, our study provides evidence that the HPA axis gene × job stress interaction may play an important role in sleep disturbances. Furthermore, according to previous research, the gene × stress interaction can be explained by the diathesis-stress model (Belsky and Pluess 2009). The model suggests that individuals with "vulnerable genes" are prone to stress-related diseases such as sleep disturbances when confronted with stress or adverse environments, while individuals with "resilient genes" will not be affected (Monroe and Simons 1991;Shao et al., 2018). As diathesis-stress research has highlighted, the interaction of FKBP5 variants with trauma and adverse environments has been found to confer risk for several psychopathological phenotypes (Zannas et al., 2016). In this study, the FKBP5 rs1360780-CC and rs9470080-CC genotypes and the CRHR1 rs110402-AA genotype may be risk genotypes susceptible to stressful environments, supporting the diathesis-stress model. Therefore, to reduce the risk of sleep disturbances, individuals with genetic susceptibility should avoid or reduce job stress as much as possible.

This study has several strengths. It is the first to examine the effects of multiple gene polymorphisms and job stress on sleep disturbances from the perspective of the HPA axis and to determine a haplotype that increases the power to detect genetic associations (Aziz et al., 2021). Furthermore, specific tests were used to investigate the pattern of gene × environment interactions (Hou et al., 2019). However, this research still has some limitations that can be addressed in future studies. First, the evaluation of sleep disturbances was entirely based on the PSQI, which is a subjective questionnaire, and it is easy to produce false positive results, which may have affected the accuracy of results. Second, there are different sources of sample bias, including reaction bias (e.g., subjects with poor sleep quality may be more inclined to complete the study than those with good sleep quality) and sample-selection bias (e.g., first-line railway workers are apt to work long hours in stressful environments). Finally, a cross-sectional design was used; thus, we could not examine the causality of the HPA axis gene × job stress interaction in the development of sleep disturbance. In future research, longitudinal designs should be used to further study this causal relationship. This study provides a reliable basis for formulating strategies to reduce employees' job stress and improve sleep quality.

Conclusions

This is the first study to investigate the effect of the interaction between job stress and HPA axis gene polymorphisms on sleep disturbances in railway frontline workers. As the main factor affecting sleep quality, job stress was found to increase the risk of sleep disturbances. The FKBP5 rs1360780-T and rs4713916-A alleles and the CRHR1 rs110402-G allele were also risk factors for sleep disturbances. More importantly, the GMDR results showed that the interactions of SNPs with job stress increased the risk of sleep disturbances, which is the core conclusion of our study. These findings provide new insight into the correlation between job stress and HPA axis gene polymorphisms and their interaction with sleep disturbances.

Acknowledgements

The authors want to express their sincere gratitude to all participants for participating in the study.

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Table 1(on next page)

Description of primer sequences

1 **Table 1 Description of primer sequences**

Gene/SNPs	Major/minor alleles	Primer (5′→3′)
FKBP5		
rs1360780	C/T	Forward: 5′-GGCATGGGCACTCTGAAAAGAT-3′ Reverse: 5′-TCTCTTGTGCCAGCAGTAGCAAGT-3′
rs3800373	A/C	Forward: 5′-GGCATGGGAAGCTGTCTTCAAC-3′ Reverse: 5′-CCAGCATTGCTACTGCTCAGCTTC-3′
rs9470080	C/T	Forward: 5′-TCTTTTCCAGGCTATGAATTGACAAA-3′ Reverse: 5′-TGTGTCCAGCCATGTGCTTTTT-3′
rs4713916	G/A	Forward: 5′-TGGCAACCCTAACCTCTCTGGA-3′ Reverse: 5′-TGTAGGTTCGGGGTACATGTGAAG-3′
rs3777747	A/G	Forward: 5′-CCGCCTAAGCCTGTTGAGAAGA-3′ Reverse: 5′-TCCAGTTGTTGGCGTACCTCCT-3′
rs9296158	G/A	Forward:5′-5CACTCGTTCTGTTATACTCATTCCATGC-3′ Reverse: 5′-AGGCCTGGGCTAGGGGTAATTC-3′
CRHR1		
rs110402	G/A	Forward: 5′-AGATCAGCGGATGGTGAAGAGG-3′ Reverse: 5′-CTTGGCTGCCTAGAACCCTGAC-3′
CRHR2		
rs2267715	A/G	Forward: 5′-TCTCTCCCAGCAGGGAAGTTGT-3′ Reverse: 5′-CTGGAGGGAGTGGGGGTAAACT-3′
NR3C1		
rs41423247	G/C	Forward: 5′-GGGGATGAGGTTACGGGGTAGA-3′ Reverse: 5′-TGCTCACAGGGTTCTTGCCATA-3′

2

Table 2(on next page)

Demographic characteristics of 671 participants in nonsleep disturbance and sleep disturbance group

Table 2 Demographic characteristics of 671 participants in nonsleep disturbance and sleep disturbance group

Variables	N	Non-sleep disturbance (%)	Sleep Disturbance (%)	χ^2	*P*-value
Gender					
Male	363	221 (60.9)	142 (39.1)	0.31	0.58
Female	308	181 (58.8)	127 (41.2)		
Age (years)					
≤30	159	95 (59.7)	64 (40.3)	3.97	0.27
31-40	234	147 (62.8)	87 (37.2)		
41-50	200	109 (54.5)	91 (45.5)		
>51	78	51 (65.4)	28 (34.6)		
Ethnicity					
Han	526	318 (60.5)	208 (39.5)	0.30	0.58
Minority	145	84 (57.9)	61 (42.1)		
Marital status					
Unmarried	118	68 (57.6)	50 (42.4)	2.04	0.36
Married	517	316 (61.1)	201 (38.9)		
Divorced or Widowed	36	18 (50.0)	18 (50.0)		
Smoking status					
Non-smoker	409	246 (60.1)	163 (39.9)	0.02	0.88
Smoker	262	156 (59.5)	106 (40.5)		
Alcohol status					
Non-drinker	310	183 (59.0)	127 (41.0)	0.19	0.67
Drinker	361	219 (60.7)	142 (39.3)		
Job stress					
Non-job stress	366	245 (60.9)	121 (45.0)	16.57	**<0.01**
Job stress	305	157 (39.1)	148 (55.0)		

Table 3(on next page)

Correlations between the job stress and sleep disturbance and its component scores (n = 671)

[a]Adjusted for gender, age, ethnicity, marital status, smoking status and alcohol status. [b]r<0 indicates negative correlation, and r>0 indicates positive correlation. [c]There were significant positive correlations between ERI and PSQI (r=0.16, $P < 0.01$).

Table 3 Correlations between the job stress and sleep disturbance and its component scores (n = 671)

Note:

[a] Adjusted for gender, age, ethnicity, marital status, smoking status and alcohol status.

		Subjective Sleep Quality	Sleep Latency	Sleep Duration	Sleep Efficiency	Sleep Disturbance	Sleep Medication	Daytime Dysfunction	PSQI
Over-commitment	R	0.01	-0.03	-0.01	-0.01	-0.02	0.12	0.00	0.08
	P	0.86	0.45	0.88	0.76	0.62	**<0.01**	0.96	**0.04**
Job effort	R	0.02	-0.03	-0.01	-0.05	0.05	0.11	0.04	-0.01
	P	0.61	0.45	0.74	0.21	0.22	**0.01**	0.26	0.84
Job reward	R	0.05	-0.02	0.00	0.01	-0.01	0.05	-0.02	0.01
	P	0.19	0.53	0.98	0.90	0.80	0.24	0.59	0.71
ERI	R	0.10	0.56	-0.15	0.03	0.03	-0.03	-0.05	0.16
	P	**0.01**	**<0.01**	**<0.01**	0.39	0.52	0.49	0.23	**<0.01**

[b] r<0 indicates negative correlation, and r>0 indicates positive correlation.

[c] There were significant positive correlations between ERI and PSQI (r=0.16, $P < 0.01$).

Table 4(on next page)

Associations of 9 HPA axis SNPs with sleep disturbances

[a] Adjusts for sex, age, race, marital status, smoking status, and alcohol status. [b] The chi-square goodness-of-fit test showed that the genotypic frequencies of HPA axis 9 SNPs in the non-sleep disturbance group and the sleep disturbance group were consistent with Hardy-Weinberg equilibrium ($P > 0.05$). $^{*}P < 0.01$.

1

2 **Table 4 Associations of 9 HPA axis SNPs with sleep disturbances**

Genes	SNPs	Genotypes &Alleles	Frequencies N (%)		OR (95%CI)	HWE	
			Non-sleep disturbance (n=402)	Sleep disturbance (n=269)		Non-sleep disturbance	Sleep disturbance
		CC	231 (57.5)	123 (45.7)	1.00	0.88	0.09
		CT	152 (37.8)	93 (34.6)	1.15 (0.82-1.61)		
	rs1360780	TT	19 (4.7)	53 (19.7)	5.24 (2.97-9.24)*		
		C allele	614 (76.4)	339 (63.0)	1.00		
		T allele	190 (23.6)	199 (37.0)	1.75 (1.38-2.22)*		
		AA	234 (58.2)	156 (58.0)	1.00	0.90	0.71
FKBP5		AC	149 (37.1)	91 (33.8)	0.92 (0.66-1.28)		
	rs3800373	CC	19 (4.7)	22 (8.2)	1.74 (0.91-3.32)		
		A allele	617 (76.7)	403 (74.9)	1.00		
		C allele	187 (23.2)	135 (25.1)	1.11 (0.86-1.43)		
		CC	187 (46.5)	140 (52.0)	1.00	0.92	0.90
	rs9470080	CT	170 (42.3)	112 (41.6)	0.88 (0.64-1.22)		
		TT	45 (11.2)	17 (6.3)	0.51 (0.28-0.92)*		

	C allele	544 (67.7)	392 (72.9)	1.00		
	T allele	260 (32.3)	146 (27.1)	0.65 (0.51-0.83)*		
	GG	256 (63.7)	152 (56.5)	1.00	0.99	0.99
	GA	130 (32.3)	99 (36.8)	1.28 (0.92-1.79)		
rs4713916	AA	16 (4.0)	18 (6.7)	1.90 (0.94-3.83)		
	G allele	642 (79.9)	403 (74.9)	1.00		
	A allele	162 (20.1)	135 (25.1)	1.68 (1.30-2.18)*		
	AA	66 (16.4)	50 (18.6)	1.00	0.77	0.81
	GA	179 (44.5)	120 (44.6)	0.89 (0.58-1.37)		
rs3777747	GG	157 (39.1)	99 (36.8)	0.83 (0.53-1.30)		
	A allele	515 (64.1)	220 (40.9)	1.00		
	G allele	289 (35.9)	318 (59.1)	1.13 (0.90-1.41)		
	GG	202 (50.2)	131 (48.7)	1.00	1.00	0.87
	GA	167 (41.5)	109 (40.5)	1.01 (0.72-1.40)		
rs9296158	AA	33 (8.2)	29 (10.8)	1.36 (0.79-2.34)		
	G allele	571 (71.0)	371 (69.0)	1.00		
	A allele	233 (29.0)	167 (31)	1.13 (0.89-1.43)		

CRHR1	rs110402	AA	316 (78.6)	201 (74.7)	1.00	0.73	0.43
		GA	78 (19.4)	57 (21.2)	1.15 (0.78-1.69)		
		GG	8 (2.0)	11 (4.1)	2.16 (0.86-5.47)		
		A allele	94 (11.7)	79 (14.7)	1.00		
		G allele	710 (88.3)	459 (85.3)	1.43 (1.09-1.87)*		
CRHR2	rs2267715	AA	79 (59.5)	61 (22.7)	1.00	0.69	0.89
		GA	183 (34.3)	127 (47.2)	0.90 (0.60-1.35)		
		GG	140 (6.2)	81 (30.1)	0.75 (0.49-1.15)		
		A allele	341 (42.4)	249 (46.3)	1.00		
		G allele	463 (57.6)	289 (53.7)	0.93 (0.74-1.15)		
NR3C1	rs41423247	GG	258 (64.2)	168 (62.5)	1.00	0.65	0.81
		GC	122 (30.3)	85 (31.6)	1.07 (0.76-1.50)		
		CC	22 (5.5)	16 (5.9)	1.12 (0.57-2.19)		
		G allele	638 (79.4)	421 (78.3)	1.00		
		C allele	166 (20.6)	117 (21.7)	1.10 (0.84-1.43)		

Note:

[a] Adjusts for sex, age, ethnicity, marital status, smoking status, and alcohol status.

[b] The chi-square goodness-of-fit test showed that the genotypic frequencies of HPA axis 9 SNPs in the non-sleep disturbance group

and the sleep disturbance group were consistent with Hardy-Weinberg equilibrium ($P > 0.05$).

* $P < 0.01$.

Table 5(on next page)

Best gene-environment interaction models, as identified by GMDR

[a]Adjusted for gender, age, ethnicity, marital status, smoking status and alcohol status.[b]The best interaction model was selected based on the balance test error of the 1/10 test sample, the accuracy of the cross-validation and *P*-value, suggest that ERI× rs1360780 ×rs947008 ×rs4713916 ×rs110402 is the best interaction model (Cross-Validation Consistency:10/10, *P*<0.01). [c]Statistically significant *P* value was denoted in bold.

Table 5 Best gene-environment interaction models, as identified by GMDR

Model	Training Accuracy (%)	Testing Accuracy (%)	Cross-Validation Consistency	*P*-value
ERI	0.58	0.56	8/10	0.17
ERI×rs1360780	0.62	0.61	10/10	**0.01**
ERI×rs1360780×rs947008	0.64	0.60	4/10	**<0.01**
ERI×rs1360780×rs947008×rs110402	0.66	0.64	10/10	**<0.01**
ERI×rs1360780×rs947008×rs4713916×rs110402	0.68	0.60	10/10	**<0.01**

Note:

[a] Adjusted for gender, age, ethnicity, marital status, smoking status and alcohol status.

[b] The best interaction model was selected based on the balance test error of the 1/10 test sample, the accuracy of the cross-validation and P-value, suggest that ERI× rs1360780 ×rs947008 ×rs4713916 ×rs110402 is the best interaction model (Cross-Validation Consistency:10/10, $P<0.01$).

[c] Statistically significant *P* value was denoted in bold.

Figure 1

The interaction model between ERI and HPA genes on sleep disturbances

The dark gray box represents the high-risk factors, and the light gray represents the low-risk factors. Bars represent the maximum likelihood estimation of case weights. In the same box, the left column is positive score and the right column is negative score. N and J denote normal and job stress (ERI>1), respectively. Among them, individuals with the rs1368780-CT, rs4713916-GG, and rs9470080-CT genotype of FKBP5 and the rs110402-AA genotype of CRHR1 had the highest risk in job stress with the highest sum score.