MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2020; 26: e924202
DOI: 10.12659/MSM.924202

Received: 2020.03.11
Accepted: 2020.05.04
Available online: 2020.06.04
Published: 2020.08.01

Effects of Occupational Stress and Circadian CLOCK Gene Polymorphism on Sleep Quality of Oil Workers in Xinjiang, China

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCDEF 1 **Li Ning***
BCDEF 2 **Lingyun Shi***
BC 1 **Ning Tao**
BC 1 **Rong Li**
BC 1 **Ting Jiang**
ABEG 1 **Jiwen Liu**

1 School of Public Health, Xinjiang Medical University, Urumqi, Xinjiang, P.R. China
2 Department of Joint Surgery, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang, P.R. China

* Li Ning and Lingyun Shi contributed equally to this work

Corresponding Author: Jiwen Liu, e-mail: nl96979@163.com

Source of support: This research was funded by Public Health and Preventive Medicine, a key discipline of the13th five-year plan in Xinjiang Uygur Autonomous Region [Grant no. 99-11091113404#] and the National Natural Science Foundation of China [Grant no. 81760581]

Background: This study investigated the effect of occupational stress and circadian clock gene polymorphism on sleep disorder of oil workers in Xinjiang, China.

Material/Methods: We enrolled 2300 Xinjiang oil workers who had been working for at least 1 year. The Chinese revised version of the Occupational Stress Questionnaire (OSI-R), the Pittsburgh Sleep Quality Index (PSQI), and General Survey Questionnaire were used. A total of 308 subjects were selected for stress hormone measurements and gene polymorphism analysis of the circadian clock genes CLOCK, PER2, and PER3.

Results: The occupational stress scores were influenced by sex, smoking, marital status, age, and work type. Different work shift groups and different professional title groups had statistically significant sleep disorder incidences ($P<0.05$). The middle and high occupational stress groups had significantly higher subjective sleep quality, total PSQI scores, daytime dysfunction factor scores, and sleep disorder than in the low occupational stress group ($P<0.05$). CLOCK gene rs1801260 locus carrying TC genotype (OR=0.412, 95% CI=0.245–0.695), and CLOCK gene rs6850524 locus carrying GC and CC genotypes decreased sleep disorder risk ($OR_1=0.357$, 95% $CI_1=0.245–0.695$; $OR_2=0.317$, 95% $CI_2=0.128–0.785$). The main factors affecting the sleep quality of oil workers were length of service, individual strain capacity, glucocorticoid levels, Per3 gene, and the rs6850524 *loci* of CLOCK gene.

Conclusions: Occupational stress has an adverse effect on the sleep quality of workers. CLOCK gene and Per3 gene may increase risk of sleep disorders.

MeSH Keywords: **Circadian Clocks • Employment • Sleep Disorders**

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/924202

3322 13 3 31

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Occupational stress refers to the harmful physiological and psychological reactions that occur when job requirements do not match the ability, coping resources, and needs of workers [1]. Moderate occupational stress can help individuals to work and live better, but excessive occupational stress can cause physical or mental damage or abnormality in workers. Occupational stress is associated with a variety of adverse disease outcomes, and is also a direct cause of cardiovascular diseases like ischemic heart disease, myocardial infarction, coronary heart disease, and hypertension, and immune system diseases [2,3]. Additionally, occupational stress can affect sleep quality and even lead to sleep disorders [4,5].

Sleep disorder refers to the abnormal length and quality of sleep, and is also a manifestation of an alternating disorder of normal sleep and arousal rhythm. One of the key roles of the circadian rhythm clock is to adjust the circadian rhythm according to changes in the external environment. The circadian rhythm is a ubiquitous life phenomenon that maintains various physiological functions of the body and regulates the body's adaptability to the internal and external environments. Studies have shown that genes involved in mammalian circadian rhythms include CLOCK, Bmal1 (Arntl), cycles (Per1, Per2, and Per3), and cryptochromin genes (Cry1 and Cry2) [6,7]. Mutations in the circadian clock genes may cause changes in the length of the circadian rhythm cycle or loss of circadian rhythm, thereby altering sleep quality [8–10].

Due to the special nature of their occupation, oil workers are exposed to a variety of occupational stressors such as job diversity, long working time, single work rhythm, difficult work, poor natural environment, relatively closed working environment, frequent shift changes, and night shifts. For example, Jiang et al. found that the incidence of sleep disorders in oilfield workers was higher and that the indicators affecting sleep quality were length of employment, occupational roles, individual stress response, and individual coping resources [11]. Thus, oil workers are prone to sleep disorders.

Therefore, we conducted an epidemiological survey of occupational stress and sleep disorders in oil workers of Xinjiang, China. The relationship among the gene polymorphism of the circadian clock genes CLOCK, PER2 and PER3, occupational stress, and sleep disorders was analyzed and discussed. Our findings may provide new ideas for exploring the mechanism of sleep disorders.

Material and Methods

Study subjects

The study subjects were oil workers from a petroleum authority in the Xinjiang Uygur Autonomous Region of China. A total of 2300 oil workers were recruited using cluster sampling method. The inclusion criteria were: age 18–60 years old and length of service was more than 1 year. The exclusion criteria were: workers with family history of mental illness, history of mental illness (e.g., depression, anxiety, bipolar affective disorder) and other serious or unstable medical diseases that can affect mental symptoms, or, workers taking medications to control sleep in the last 6 months. Informed consent was obtained from each subject. This study was approved by Xinjiang Medical University.

Questionnaires

Questionnaires were used to investigate the occupational stress and sleep disorder of oil workers.

General survey

The basic information was collected from the general survey, such as sex, age, ethnicity, marital status, education level, monthly income, length of service, work type, and professional title.

Occupational stress survey

The OSI-R (Occupation Stress Inventory Revised Edition), including ORQ (Occupational Role Questionnaire), the PSQ (Personal Strain Questionnaire), and the PQQ (Personal Resources Questionnaire), was used [12,13]. It contains 14 items with 140 entries. The 140 entries were graded using a 5-level scale to assess the occupational stress and stress levels. The higher the score of occupational role and personal strain, the higher the degree of stress; the higher the personal resource score, the lower the degree of stress. According to the total score of the ORQ, the subjects were divided into 3 groups: workers with total score >180 were assigned to the high-stress group, workers with total score 140–180 was assigned to the moderate-stress group, and workers with total score <140 were assigned to the low-stress group.

Sleep quality survey

The PSQI (Pittsburgh Sleep Quality Index) was used for evaluation [14]. PSQI includes 19 self-evaluations and 5 other evaluation items. The 19th self-evaluation item and 5 other evaluation items were not used in scoring. The items for scoring included use of sleep-inducing drugs, subjective sleep quality,

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 1. The sequences of primers.

Gene	Locus	The sequence of primers (5'-3')
CLOCK	rs1801260	F: TCCACGAGTTTCATGAGATCG
		R: GAGGTCATTTCATACGTGACG
	rs6850524	F: CCCCAAATACTTGAAGATTA
		R: CTGACACCATCGCTGGTTAA
Per2	rs2304672	F: GTCGGTGTCGTTGTTAATCG
		R: TCCTTGGTGGGGTTACTGG
Per3		F: TGTCTTTTCATGTCGCCTTACTT
		R: TGTCTGCGATTGGAGTTTGA

Table 2. The PCR condition of each gene.

Gene	Locus	Cycle	Further extension
CLOCK	rs1801260	94°C 40 s, 54°C 30 s, 72°C 60 s	72°C 5 min
		35 cycles	
	rs6850524	94°C 40 s, 54°C 60 s, 72°C 60 s	72°C 5 min
		35 cycles	
Per2	rs2304672	94°C 45 s, 59°C 45 s, 72°C 45 s	72°C 7 min
		30 cycles	
Per3		94°C 40 s, 58°C 30 s, 72°C 40 s	72°C 12 min
		35 cycles	

sleep latency, sleep duration, sleep disorder, daytime dysfunction, and sleep efficiency. A 0–3 scale was used, and the total score of PSQI ranged from 0 to 21. Higher scores suggest worse sleep quality. Based on the domestic norm standard, the threshold for defining sleep disorder is a PSQI score of 7 [15].

Blood sample collection

At the same time as the questionnaire was issued, we collected blood samples. After centrifugation, serum and plasma were isolated.

ELISA

The level of cortisol in serum was determined by a radioimmunoassay assay kit (Beijing North Institute of Biotechnology Co., Beijing, China) on an automatic RIA instrument (Anhui USTC Zonkia Scientific instruments Co., Hefei, China). Melatonin and glucocorticoids levels in serum were determined by use of corresponding ELISA kits (Beijing North Institute of Biotechnology Co.).

Genome DNA extraction

The genome DNA was extracted from blood using a whole-blood genomic DNA extraction kit (solution type) (BioTeke Co., Beijing, China), and the DNA was quantitatively detected by an ultra-micro spectrophotometer. The DNA sample concentration ranged from 300 to 800 μg/μL, and the purity ranged from 1.6 to 1.9.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RELP)

The primers of CLOCK gene rs1801260 and rs6850524, Per2 gene rs2304672, and Per3 gene were synthesized and their sequences are shown in Table 1. The PCR reaction system was as follows: ddH_2O 8.5 uL, DNA template 2 uL, 2×Taq PCR Master Mix 12.5 uL, 1 uL of upstream and downstream primers. The PCR reaction condition for each gene is listed in Table 2. The PCR products were detected by electrophoresis using 1.5% agarose gel, and observed by a ZF-268 gel imaging system. The band of the Per3 gene could be directly observed after electrophoresis, and no further digestion was required. The other genes were digested using the following enzymatic system: ddH_2O 18 uL, PCR amplification product 10 uL, 10×FastDigest Buffer 2 uL, and enzyme 1 uL (Table 3). The genotyping and product length are shown in Table 4.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 3. The list of endonuclease enzyme reaction condition.

Gene	rs1801260	rs6850524	rs2304672
Endonuclease	Bsp12861	Bsm I	HpyCH4V
Water bath	37°C 16 h	37°C 5 min	37°C 5 min
Electrophoresis gel	2.5% agarose gel	2.5% agarose gel	8% polyacrylamide gel
Voltage	70 V	80 V	120 V

Table 4. The enzymatic fragment type of the PCR products.

Gene	Locus	Allele	Type	Fragment length (bp)
CLOCK	rs1801260	C/T	TT	221
			CC	221, 125, 96
			CT	125, 96
	rs6850524	C/G	CC	490
			GC	490, 308, 182
			GG	308,182
Per2	rs2304672	C/G	CC	114
			CG	114, 61,53
			GG	61,53

Statistical analysis

SPSS 17.0 software (SPSS, Inc., USA) was used. Measurement data are expressed as mean ± standard deviation. Comparison between 2 groups was analyzed by *t* test. Analysis of variance was used for comparison of multiple groups, followed by LSD test. The rank sum test was used for comparison when there was heterogeneity of variance. The rates were compared using the chi-square test. Multivariate analysis was performed using logistic regression analysis. The odds ratio (OR) of genetic polymorphisms to sleep disorder risk and its 95% confidence interval (CI) were calculated. A *P* value less than 0.05 was considered as statistically significant.

Results

Demographic characteristics of oil workers from Xinjiang oilfield

A total of 2116 valid questionnaires were finally collected. The demographic characteristics of the 2116 subjects are shown in Table 5. Among them, 53.02% were aged 30–45 years old, 73.72% were of Han ethnicity, 87% were married, and 62.85% were college graduates or above (Table 5).

Comparison of the OSI-R scores of oil workers in Xinjiang with domestic norm scores

The occupational stress level of Xinjiang oilfield workers was compared with the domestic norms of occupational stress. The scores of the ORQ and the PSQ of the oil workers in Xinjiang, China were higher than that of the domestic norm [16], and the scores of PRQ were lower than that of the domestic norm ($P<0.05$, Table 6). These indicate that oilfield workers have higher levels of occupational stress.

Comparison of occupational stress scores of oil workers with different demographic characteristics

To understand the overall score of the occupational stress scale of the oilfield workers, this study compared the scores of occupational stress among different demographic groups at 3 levels. The occupational role scores of males (174.80±25.81) and smokers (174.51±26.82) were higher than those of females (170.51±23.12) and non-smokers (171.57±23.22), and the differences were statistically significant ($P<0.05$). The personal nervous response scores of married individuals (106.62±16.79) were higher than those of unmarried persons (104.37±16.39) ($P<0.05$). The scores of personal resources of individuals aged >45 years old (126.80±21.47) were higher than in those aged 30–45 years old (124.06±20.28) and ≤30 years old (125.46±20.36) ($P<0.05$). The scores of personal resources of the stokers (124.17±18.24) were higher than those of the other 2 work types (123.32±21.67

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 5. Demographic characteristics of 2116 oil workers.

Items		Cases	Ratio (%)
Sex	Male	1020	48.20
	Female	1096	51.80
Age	≤30 years old	385	18.19
	30~45 years old	1122	53.02
	>45 years old	609	28.78
Ethnic group	Han	1560	73.72
	Minority	556	26.28
Length of service	≤15 years	765	36.15
	>15 years	1351	63.85
Work type	Oil production	253	11.96
	Oil delivery	737	34.83
	Stoker	1126	53.21
Work shifts	Fixed day shift	775	36.63
	Regular shift	1180	55.77
	Irregular shift	161	7.61
Professional titles	Medium grade and below	1208	57.09
	Senior	908	42.91
Education	Below junior college	786	37.15
	Junior college and above	1330	62.85
Marriage status	Unmarried	275	13.00
	Married	1841	87.00
Income	≤3,500	733	34.64
	>3,500	1383	65.36
Smoking	Yes	722	34.12
	No	1394	65.88
Drinking	Yes	1112	52.55
	No	1004	47.45

Table 6. Comparison of OSI-R scores between domestic norm and oil workers in Xinjiang.

Items	Oil workers in Xinjiang	Domestic norm	t	P
Occupational role questionnaire (ORQ)	172.57±24.54	162.89±27.04	18.153	<0.001
Personal strain questionnaire (PSQ)	106.33±16.75	91.01±17.19	42.065	<0.001
Personal resources questionnaire (PRQ)	125.10±20.67	129.23±17.73	–9.184	<0.001

and 124.17±18.24, respectively) (P<0.05; Table 7). These indicate that sex, smoking, marital status, age, and work type can affect the score of occupational stress.

Comparison of different occupational stress groups

The influence of different demographic characteristics on occupational stress was analyzed. Different occupational stress groups had significant differences in sex, type of work, and smoking status (P<0.05) (Table 8). Occupational stress of males was higher than that of females, and stokers had higher occupational stress than oil production workers and delivery workers. Non-smokers had more stress than smokers. These indicate that males, stokers, and smokers have high occupational stress.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 7. Comparison of occupational stress score in the population with different demographic characteristics.

Items		Occupational role			Personal strain			Personal resources		
			$t/F/\chi^2$	*P*		*t/F*	*P*		$t/F/\chi^2$	*P*
Sex	Male (n=1020)	174.80±25.81	4.017	**0.000**	106.42±17.01	0.252	0.801	124.59±20.95	−1.098	0.272
	Female (n=1096)	170.51±23.12			106.24±16.51			125.58±20.95		
Age	≤30 years old (n=385)	173.54±23.08	0.450	0.638	105.82±15.67	0.421	0.656	125.46±20.36	3.558	**0.029**
	30~45 years old (n=1122)	172.18±24.39[#]			106.63±17.07			124.06±20.28		
	>45 years old (n=609)	172.70±25.70			106.09±16.83			126.80±21.47		
Ethnic group	Han (n=1560)	172.2±24.84	−0.962	0.336	106.72±16.80	1.806	0.071	124.92±20.84	−0.675	0.500
	Minority (n=556)	173.43±23.69			105.23±16.58			125.61±20.18		
Education	Below junior college (n=786)	172.00±25.10	−0.824	0.410	105.64±16.13	−1.460	0.144	124.22±20.12	−1.505	0.132
	Junior college and above (n=1330)	172.91±24.21			106.74±17.10			125.62±20.97		
Marriage status	Unmarried (n=275)	173.70±21.43	0.814	0.416	104.37±16.39	−2.078	**0.038**	124.50±20.62	−0.517	0.605
	Married (n=1841)	172.41±24.97			106.62±16.79			125.19±20.68		
Income	≤3500 (n=733)	172.85±24.86	0.382	0.703	106.78±16.14	0.900	0.368	125.05±20.77	−0.084	0.933
	>3500 (n=1383)	172.43±24.38			106.09±17.07			125.13±20.62		
Length of service	≤15 years (n=765)	173.07±23.92	0.693	0.489	106.42±16.16	0.198	0.843	124.96±20.43	−0.246	0.806
	>15 years (n=1351)	172.30±24.89			106.27±17.08			125.19±20.81		
Work type	Oil production (n=253)	171.18±27.15	0.707	0.702	105.95±14.81	0.977	0.377	123.32±21.67	6.791	**0.034**
	Oil delivery (n=737)	173.34±21.20[##]			105.73±16.89			124.17±18.24		
	Stoker (n=1126)	172.38±25.93			106.80±17.07			126.11±21.86		
Professional titles	Medium grade and below (n=1208)	172.17±23.73	−0.864	0.388	106.73±16.63	1.289	0.198	125.46±20.69	0.916	0.360
	Senior (n=908)	173.11±25.58			105.79±16.90			124.63±20.64		
Work shifts	Fixed day shift (n=775)	174.00±25.28	2.378	0.093	105.95±16.60	0.346	0.708	126.19±20.58	2.375	0.093
	Regular shift (n=1180)	171.56±24.21			106.50±16.64			124.23±20.87		
	Irregular shift (n=161)	173.18±23.10			106.88±18.33			126.27±19.42		
Smoking	Yes (n=722)	174.51±26.82	2.495	**0.013**	106.28±16.58	−0.094	0.925	123.98±20.95	−1.793	0.073
	No (n=1394)	171.57±23.22			106.35±16.85			125.68±20.51		
Drinking	Yes (n=1112)	173.29±24.87	1.404	0.160	106.52±16.57	0.587	0.396	124.55±20.40	0.197	0.900
	No (n=1004)	171.79±24.16			106.12±16.96			125.71±20.95		

[#] *P*<0.05, comparison between 30~45 years old and >45 years old groups; [##] *P*<0.05, comparison with the moderate-stress group of oil delivery worker and stoker.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 8. Comparison between different occupational stress groups with different demographic characteristics.

	Item	Low-stress group (n=152)		Moderate-stress group (n=1213)		High-stress group (n=751)		χ^2	I
Sex	Male	78	(3.69%)	537	(25.38%)	405	(19.14%)	17.962	<0.001
	Female	74	(3.50%)	676	(31.95%)	346	(16.35%)		
Age	≤30 years old	24	(1.13%)	212	(10.02%)	149	(7.04%)	8.478	0.076
	30~45 years old	80	(3.78%)	673	(31.81%)	369	(17.44%)		
	>45 years old	48	(2.27%)	328	(15.50%)	233	(11.01%)		
Ethnic group	Han	108	(5.10%)	911	(43.05%)	541	(25.57%)	2.854	0.240
	Minority	44	(2.08%)	302	(14.27%)	210	(9.92%)		
Length of service	≤15 years	52	(2.46%)	436	(20.60%)	277	(13.09%)	0.445	0.800
	>15 years	100	(4.73%)	777	(36.72%)	474	(22.40%)		
Work type	Oil production	22	(1.04%)	147	(6.95%)	84	(3.97%)	12.226	0.016
	Oil delivery	35	(1.65%)	445	(21.03%)	257	(12.15%)		
	Stoker	95	(4.49%)	621	(29.35%)	410	(19.38%)		
Work shifts	Fixed day shift	52	(2.46%)	432	(20.42%)	291	(13.75%)	3.458	0.484
	Regular shift	86	(4.06%)	693	(32.75%)	401	(18.95%)		
	Irregular shift	14	(0.66%)	88.00	(4.16%)	59	(2.79%)		
Professional titles	Medium grade and below	87	(4.11%)	703	(33.22%)	418	(19.75%)	1.000	0.607
	Senior	65	(3.07%)	510	(24.10%)	333	(15.74%)		
Education	Below junior college	67	(3.17%)	437	(20.65%)	282	(13.33%)	3.833	0.147
	Junior college and above	85	(4.02%)	776	(36.67%)	469	(22.16%)		
Marriage status	Unmarried	16	(0.76%)	158	(7.47%)	101	(4.77%)	0.957	0.620
	Married	136	(6.43%)	1055	(49.86%)	650	(30.72%)		
Income	≤3500	52	(2.46%)	415	(19.61%)	266	(12.57%)	0.312	0.856
	>3500	100	(4.73%)	798	(37.71%)	485	(22.92%)		
Smoking	Yes	60	(2.84%)	375	(17.72%)	287	(13.56%)	13.085	0.001
	No	92	(4.35%)	838	(39.60%)	464	(21.93%)		
Drinking	Yes	80	(3.78%)	619	(29.25%)	413	(19.52%)	2.922	0.232
	No	72	(3.40%)	594	(28.07%)	338	(15.97%)		

Comparison of sleep disorder incidence of oil workers in Xinjiang with different demographic characteristics

To understand the occurrence and distribution of sleep disorders in the 2116 oil workers in detail, the sleep disorder incidence of different demographic groups were compared. Among them, the incidence of sleep disorder was 36.67% (776/2116). The incidence of sleep disorder in male workers was 35.00%, and the incidence of sleep disorder in female workers was 38.23%. The incidence of sleep disorder among oil workers with different shifts and professional titles was significantly different (*P*<0.05; Table 9). These indicate that shift work and personal positions can affect sleep quality, which can lead to sleep disorders.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 9. Comparison of sleep disorder incidence rate of oil workers in Xinjiang with different characteristics.

Items		Cases	Sleep disorder (n=776)	χ^2	*P*
Sex	Male	1020	357 (35.00%)	2.373	0.123
	Female	1096	419 (38.23%)		
Age	≤30 years old	385	131 (34.03%)	3.242	0.198
	30~45 years old	1122	431 (38.41%)		
	>45 years old	609	214 (35.14%)		
Ethnic group	Han	1560	556 (36.28%)	0.391	0.532
	Minority	556	210 (37.77%)		
Length of service	≤15 years	765	269 (35.16%)	1.176	0.278
	>15 years	1351	507 (37.53%)		
Work type	Oil production	253	91 (35.97%)	4.330	0.115
	Oil delivery	737	292 (39.62%)		
	Stoker	1126	393 (34.90%)		
Work shifts	Fixed day shift	775	243 (31.35%)	17.937	**0.000**
	Regular shift	1180	459 (38.90%)		
	Irregular shift	161	74 (45.96%)		
Professional title	Medium grade and below	1208	411 (34.02%)	8.511	**0.004**
	Senior	908	365 (40.20%)		
Education	Below junior college	786	300 (38.17%)	1.203	0.273
	Junior college and above	1330	476 (35.79%)		
Marriage	Unmarried	275	92 (33.45%)	1.410	0.235
	Married	1841	684 (37.15%)		
Income	≤3500	733	262 (35.74%)	0.417	0.518
	>3500	1383	514 (37.17%)		
Smoking	Yes	722	283 (39.20%)	3.006	0.083
	No	1394	493 (35.37%)		
Drinking	Yes	1112	424 (38.13%)	2.141	0.143
	No	1004	352 (35.06%)		

Assessment of sleep quality in Xinjiang oil workers with different occupational stress levels

As shown in Table 10, sleep disorder, PSQI total score, daytime dysfunction, and subjective sleep quality of oil workers were statistically different among oil workers with different occupational stress levels (*P*<0.01). These results indicate that low quality of sleep is usually accompanied by high occupational stress.

Gene polymorphism analysis of circadian clock genes

Hardy-Weinberg genetic balance test of gene locus

T determine whether the CLOCK, Per2, and Per3 *loci* in this study conform to the H-W law, a genetic balance test on 4 *loci* of the 3 genes was performed. CLOCK gene rs1801260 and rs6850524 *loci* and Per2 gene rs2304672 locus and Per3 gene had no significant difference in polymorphism (*P*>0.05). The results indicate that the subjects are representative of the population and the sample size is sufficient for the subsequent studies (Table 11, Figure 1).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 10. Comparison of the sleep quality of Xinjiang oil workers in different occupational stress groups.

Index	Low-stress group	Medium stress group	High-stress group	F/χ^2	*P*
Cases	152	1213	751	–	–
PSQI score	5.44±3.53	6.40±3.73#	6.70±4.19#	11.037	**0.004**
Subjective sleep quality	0.95±0.87	1.24±0.87#	1.24±0.93#	14.911	**0.001**
Sleep latency	1.18±0.89	1.35±0.96	1.37±0.98	4.655	0.098
Sleeping duration	1.05±0.94	1.07±0.93	1.16±0.96	2.502	0.082
Sleep efficiency	0.24±0.61	0.20±0.49	0.26±0.58	3.921	0.141
Sleep disorder	0.89±0.69	1.12±0.72#	1.13±0.72#	7.315	0.001
Hypnotic drugs	0.23±0.58	0.26±0.63	0.31±0.70	2.442	0.295
Daytime dysfunction	0.90±0.82	1.16±0.91#	1.23±0.98#	14.729	**0.001**

# *P*<0.05, compared with the low-stress group.

Table 11. Hardy-Weinberg genetic equilibrium test of gene locus.

Gene	Genotype	Sleep disorder-positive group		χ^2	*P*	Sleep disorder-negative group		χ^2	*P*
		Actual value	Expected value			Actual value	Expected value		
CLOCK	rs1801260								
	TT	107	110.57	0.430	0.512	78	80.01	0.645	0.422
	TC	39	57.86			66	61.99		
	CC	8	7.57			10	12.01		
	rs6850524								
	GG	81	80.01	0.158	0.691	57	62.36	3.489	0.062
	GC	60	61.99			82	71.27		
	CC	13	12.01			15	20.36		
Per2	rs2304672								
	CC	134	134.65	0.743	0.389	127	128.18	1.422	0.233
	CG	20	18.70			27	24.63		
	6GG	0	0.65			0	1.18		
Per3	4/4	111	108.06	3.038	0.081	129	127.27	2.836	0.092
	4/5	36	41.88			22	25.45		
	5/5	7	4.06			3	1.27		

The genotype and allele frequency distribution of CLOCK, Per2, and Per3 among the workers with different sleep conditions

The distribution of CLOCK, Per2, and Per3 genotypes among different sleep quality groups was analyzed. In the analysis of CLOCK, Per2, and Per3 genotype frequencies and allele frequency distribution, rs1801260, rs6850524, and Per3 genotypes and alleles distributed in the sleep disorder-positive and -negative groups were statistically different (*P*<0.05). However, there was no significant difference in the distribution between the genotypes and alleles of Per2 gene rs2304672 locus (>0.05).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

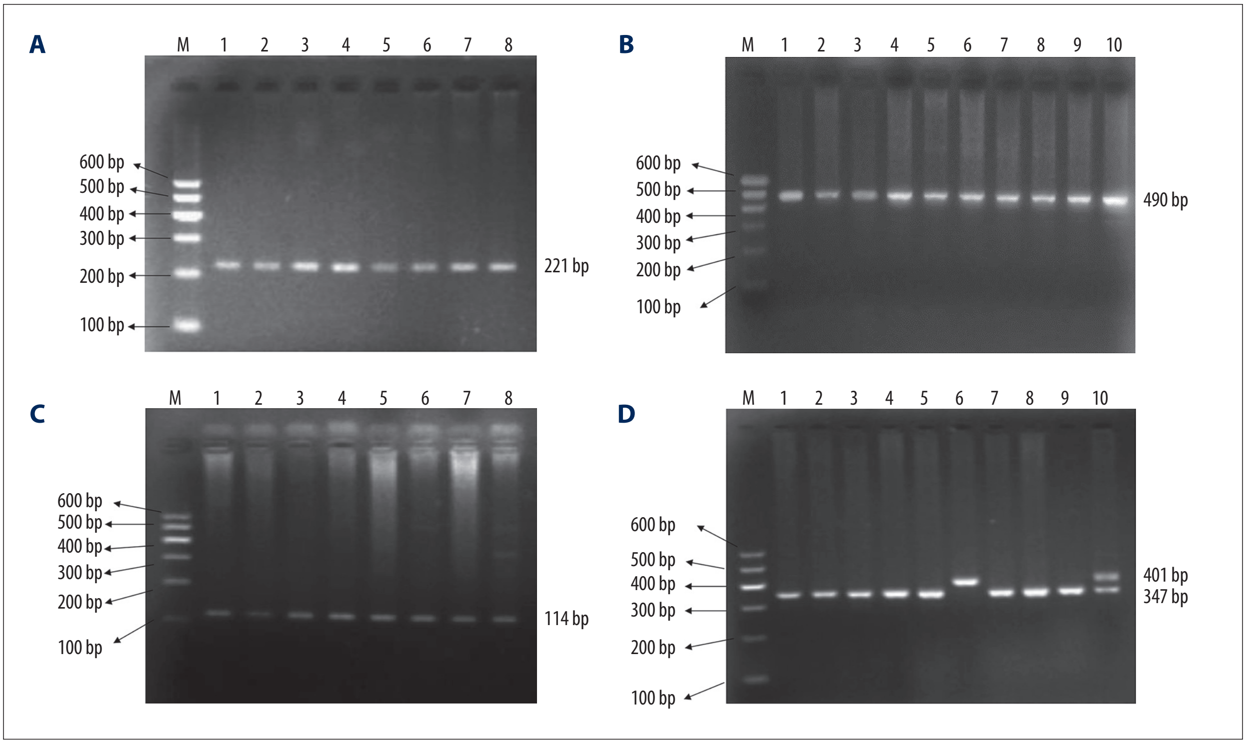

Figure 1. The electrophoresis results of the PCR products of (**A**) rs1801260, (**B**) rs6850524, (**C**) rs2304672, and (**D**) Per3. For the Per3 gene, Lanes 1–5 and 7–9 were 4/4 genotype, Lane 6 was 5/5 genotype, and Lane 10 was 4/5 genotype.

These results suggest that the CLOCK gene rs1801260 and rs6850524 *loci* and Per3 genes are involved in the development of sleep disorder, and the Per2 gene rs2304672 locus is not associated with sleep disorder (Table 12, Figure 2).

Analysis of the risk of sleep disorder caused by CLOCK, Per2, and Per3

To analyze the relationship between CLOCK, Per2, and Per3 genes and sleep disorder, multivariate unconditional logistic regression analysis was performed. The CLOCK gene rs1801260 locus TC genotype was a protective factor for sleep disorder (95% CI=0.263-0.704, OR=0.431) in reference to the TT genotype (Table 13). The CLOCK gene rs6850524 GC genotype was a protective factor for sleep disorder (95% CI=0.320-0.828, OR=0.515), in reference to the GG genotype. The Per3 gene was referenced to the 4/4 genotype, and the risk of sleep disorder development in the population with 4/5 genotype was 1.902 times higher than in the population carrying the 4/4 genotype (95% CI=1.056–3.424). After adjusting for personal strain and occupational stress level, the CLOCK gene rs1801260 locus carrying the TC genotype reduced the risk of sleep disorder (OR=0.412, 95% CI=0.245–0.695). CLOCK gene rs6850524 locus carrying GC and CC genotypes reduced the risk of sleep disorder (OR_1=0.357, 95% CI_1=0.245–0.695; OR_2=0.317, 95% CI_2=0.128–0.785). The results suggest that the CLOCK gene rs1801260 locus TC genotype and rs6850524 locus GC and CC genotypes are protective factors for sleep disorder, which can reduce the risk of sleep disorder.

Comparison of the hormone levels

To objectively reflect the relationship of occupational stress with sleep disorders, 2 stress hormones (cortisol and glucocorticoids) and sleep-inducing hormones (melatonin) in different sleep disorder groups were compared. The sleep disorder-positive group had significantly lower glucocorticoid levels than in the sleep disorder-negative group (P<0.05). The sleep disorder-positive group had lower cortisol and melatonin levels than sleep disorder-negative group, but the difference was not significant (P>0.05; Figure 3). This suggests that a decrease in the concentration of glucocorticoids increases the risk of sleep disorder.

Discussion

The results of this study showed that Xinjiang oil workers have higher occupational stress compared with the domestic norm. This is related to the working environment of oil workers. Due to the geographical characteristics of Xinjiang, the summer is arid and hot. The maximum temperature can reach 50°C in summer, and the minimum temperature can reach –40°C in winter. The daylight time is long, the daily radiation is strong,

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 12. The distribution of CLOCK, Per2, and Per3 genotypes and alleles among populations with different sleep states.

Gene	Genotype/allele type	Sleep disorder-positive (%)		Sleep disorder-negative (%)		χ^2	*P*
CLOCK	TT	107	(69.48)	78	(50.65)	11.711	0.003
rs1801260	TC	39	(25.32)	66	(42.86)		
	CC	8	(5.19)	10	(6.49)		
	T	253	(82.14)	222	(72.08)		0.905
	C	55	(17.86)	86	(27.92)		
CLOCK	GG	81	(52.60)	57	(37.01)	7.725	**0.021**
rs6850524	GC	60	(38.96)	82	(53.25)		
	CC	13	(8.44)	15	(9.74)		
	G	222	(72.08)	196	(63.64)	5.031	**0.025**
	C	86	(27.92)	112	(36.36)		
Per2	CC	134	(87.01)	127	(82.47)	1.230	0.267
rs2304672	CG	20	(12.99)	27	(17.53)		
	GG	0	(0.00)	0	(0.00)		
	C	288	(93.51)	281	(91.23)	1.129	0.288
	G	20	(6.49)	27	(8.77)		
Per3	4/4	111	(72.08)	129	(83.77)	6.329	**0.042**
	4/5	36	(23.38)	22	(14.29)		
	5/5	7	(4.55)	3	(1.95)		
	4	258	(83.77)	280	(90.91)	7.105	0.008
	5	50	(16.23)	28	(9.91)		

the wind is dry, and the natural environment is harsh. In addition, single work content, heavy work task, and frequent shift changes are the main sources of occupational stress. Among the 2116 oil workers, the occupational role score of males was higher than that of females. This may be because male workers not only bear the pressure from work, but also bear pressure from society and family. This may make men more prone to occupational stress. In addition, the occupational role score of smokers was higher than that of non-smokers, which is consistent with the results of Jiang et al. [17]. This may because oil workers with greater occupational stress relieve the work stress by smoking. The results of this study showed that oil workers older than 45 years have higher coping ability, which may be because they have more work experience, and have become tolerant of the harsh working conditions and high-intensity tasks. They can more easily cope with problems that arise during work. Additionally, the results showed that the individual responding resource of stokers was higher than that of oil delivery workers. The possible reason may be that the work environment of stokers is indoors. Although stokers also perform shift work, they have more time to be with family and friends and their work mood is relatively better. Thus, they are better able to deal with work stress.

Sleep disorder refers to various dysfunctions during the sleep-wake cycle, including difficulty falling asleep, shallow sleep, easily waking, waking early, dreams, nightmares, no sleep sensation, and not feeling refreshed after sleep [18]. This study showed that the PSQI score of Xinjiang oil workers was 6.44±3.90, and prevalence of sleep disorder was 36.67%. This result is inconsistent with the previous report by Chien et al. [19], which may be due to the different cutoff score of the PSQI questionnaire. In addition, the prevalence of sleep disorder in oil workers with senior titles was higher than that of the workers with medium- and lower-ranking titles. Oil workers with senior titles mainly engaged in mental work, which mostly involves decision-making. Thus, their mental stress is relatively high and sleep disorder easily occurs.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

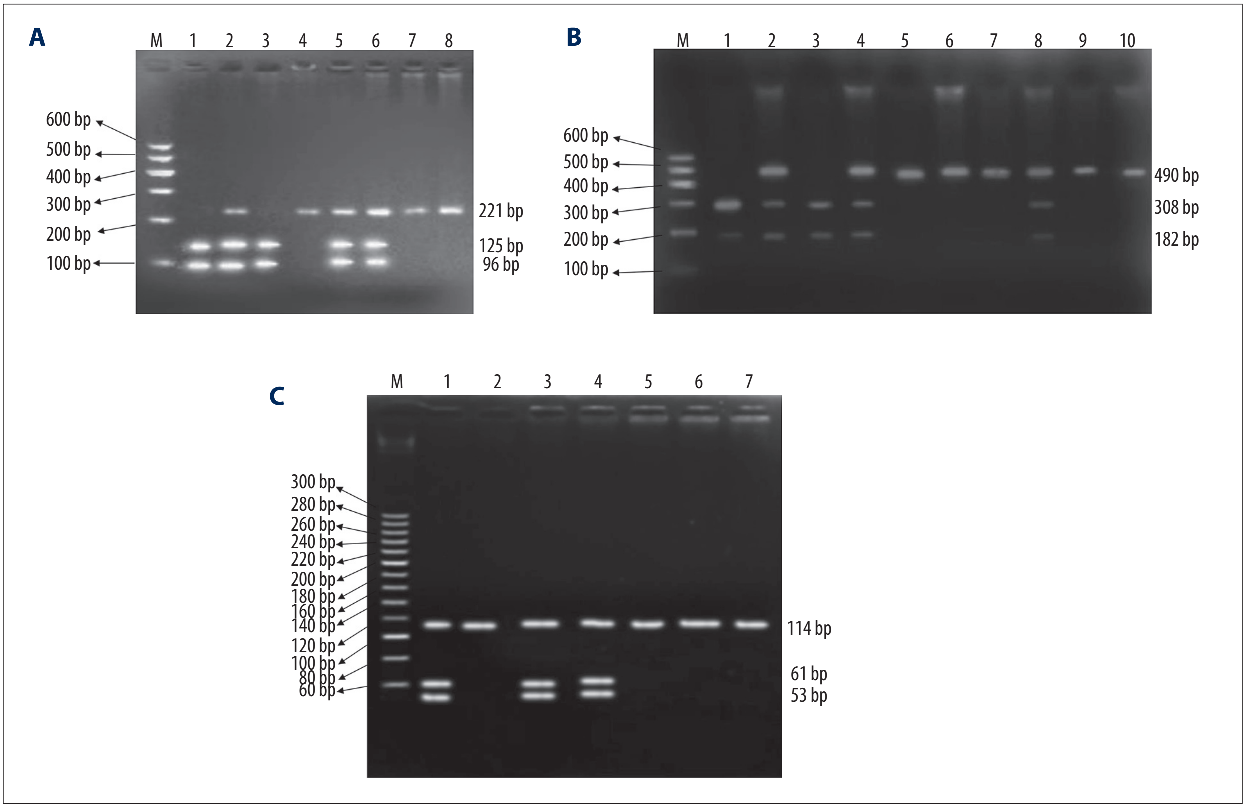

Figure 2. The electrophoresis results for restriction endonuclease digestion. (**A**) rs1801260; Lanes 1 and 3 were CT genotype, Lanes 2, 5, and 6 were CC genotype, and Lanes 4, 7, and 8 were TT genotype. (**B**) rs6850524; Lanes 1 and 3 were GG genotype, Lanes 2, 4, and 8 were GC genotype, and Lanes 5–7 and 9–10 were CC genotype. (**C**) rs2304672; Lanes 1, 3, and 4 were CG genotype, and Lanes 2 and 5–7 were CC genotype.

The results of this survey showed that daytime dysfunction, PSQI total score, sleep disorder, and subjective sleep quality were statistically significant among different occupational stress groups. After pairwise comparison, the scores of the 4 items were higher in the high- and moderate-stress groups than those in the low-stress group. This result is similar with that by Nomura et al. [20], indicating that occupational stress is related with sleep disorder. However, the causal relationship between occupational stress and sleep quality were not explained, which needs further investigation.

The circadian rhythm is a 24-hour period physiological phenomenon regulated by the endogenous circadian clock genes. Studies [21,22] have shown that sleep disorder is closely related to these circadian clock genes. This study found that the CLOCK gene rs1801260 and rs6850524 *loci* could reduce the incidence of sleep disorder. The C allele frequency of the CLOCK gene rs6850524 locus was lower in the sleep disorder-positive group, suggesting that C allele acts as susceptible allele to affect the sleep status of oil workers, and is a protective factor for the occurrence of sleep disorder. Consistently, Lou et al. [23] found that the CLOCK rs1801260 polymorphism may be an independent risk factor for sleep disorder in Parkinson's patients. It is also reported that the rs1801260 C allele frequency was significantly higher in children with attention deficit hyperactivity disorder who has sleep disorder than in those without sleep disorder [24]. In addition, studies showed that the 3111tnc variant of CLOCK gene rs1801260 could affect daytime preference. The CLOCK gene rs1801260 CC site was associated with nocturnal preference and delayed sleep episodes, and the carriers had shorter sleep duration compared with the subjects with CLOCK gene rs1801260 T site [8,25]. This conclusion is similar to this study. Antypa et al. [26] showed that 3111C/C genotype polymorphism of CLOCK gene and environmental stress increase could affect women's sleep. In a large-sample cohort study by Serretti et al., it was found that the polymorphism of the CLOCK3111T/C locus was associated with sleep disorder in people with depression, and TC and CC alleles carriers were more likely to have problems like difficulty falling asleep, waking up early, and poor sleep maintenance [27]. However, in another cohort study, TC and CC variant alleles were not associated with insomnia [28].

Cortisol and glucocorticoid secreted by the adrenal gland are important stress hormones in the body. Melatonin can maintain the "sleep-wake cycle", that is, normal nighttime sleep state and daytime waking state. It is an important neurohormone

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 13. The risk analysis of CLOCK, Per2, and Per3 genotypes in sleep disorder.

Gene	Genotype/allele type	OR (95% CI)	OR# (95% CI)
CLOCK	rs1801260		
	TT	1	1
	TC	0.431 (0.263–0.704)**	0.412 (0.245–0.695)**
	CC	0.583 (0.220–1.545)	0.445 (0.158–1.257)
CLOCK	rs6850524		
	GG	1	1
	GC	0.515 (0.320–0.828)**	0.357 (0.245–0.695)**
	CC	0.610 (0.270–1.380)	0.317 (0.128–0.785)*
Per2	rs2304672		
	CC	1	1
	CG	0.702 (0.375–1.314)	0.617 (0.317–1.202)
	GG	0	0
Per3	4/4	1	1
	4/5	1.902 (1.056–3.424)*	1.691 (0.912–3.135)
	5/5	2.712 (0.685–10.737)	1.676 (0.392–7.155)

OR# – after adjusting individual stress and occupational stress levels. * $P<0.05$, ** $P<0.01$.

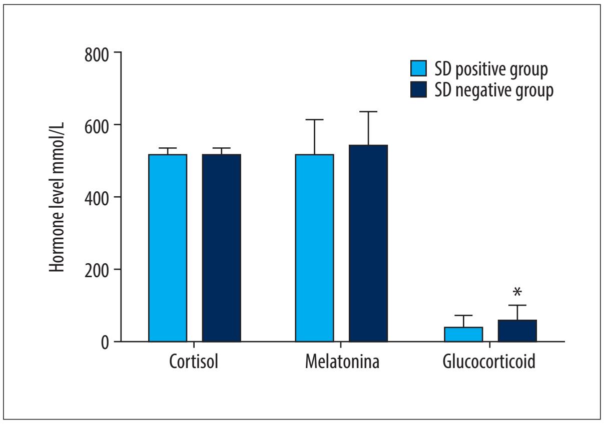

Figure 3. The hormone levels in different groups. * $P<0.05$, comparison between the 2 groups.

regulating the biological rhythm of the human body [29]. The hypothalamic-pituitary-adrenal axis (HPA) has a bidirectional regulation on the secretion of cortisol. The release of cortisol is directly regulated by the binding of melatonin to the melatonin receptors at multiple levels of the HPA axis, while the secretion of the hormone by the pineal body is regulated by negative feedback of the HPA axis. There is a close relationship between cortisol and melatonin [30]. Here, we found that the concentrations of cortisol and melatonin in the sleep disorder-positive group were decreased, but the difference was not significant. Although their secretion had a circadian rhythm, the blood was drawn in the morning physical examination, which may have affected the concentrations of melatonin and cortisol in the blood of workers. The sleep disorder-positive group had lower glucocorticoids than in the sleep disorder-negative group, suggesting that the increase in glucocorticoid levels reduced the incidence of sleep disorder. This result is consistent with research by Wang et al., whose results suggested that glucocorticoids combined with montelukast sodium improved sleep quality of children who had mild obstructive sleep apnea syndrome [31]. Therefore, serum glucocorticoid of oilfield workers may be used to predict the development of sleep disorder.

This study has some limitations. For example, sleep disorder was defined based on PSQI score, but not an objective indicator. Further studies with objective indicators (such as VEEG) are warranted.

Conclusions

In conclusion, the level of occupational stress is significantly correlated with sleep quality. As the intensity of occupational stress increases, the sleep disorder of oil workers tends to increase. The CLOCK genes rs1801260, rs6850524, and Per3 are susceptibility genes for sleep disorders. Glucocorticoid levels can be used as predictors of health impairment in subjects with sleep disorders.

Conflicts of interest

None.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

References:

1. Dai JM, Fu H: Advances in the assessment method of occupational stress. Journal of Environmental & Occupational Medicine, 2006; 23: 278–81
2. Wu WT, Tsai SS, Wang CC et al: Professional driver's job stress and 8-year risk of cardiovascular disease: The Taiwan bus driver cohort study. Epidemiology, 2019; 30(Suppl. 1): S39–47
3. Zaitseva NS, Siziakina LP: The influence of acute professional stress on the immune system. Allergy, 2018; 73: 469
4. Iwasaki S, Deguchi Y, Inoue K: Association between work role stressors and sleep quality. Occup Med (Lond), 2018; 68: 171–76
5. Zare R, Choobineh A, Keshavarzi S: Association of amplitude and stability of circadian rhythm, sleep quality, and occupational stress with sickness absence among a gas company employees – a cross sectional study from Iran. Saf Health Work, 2017; 8: 276–81
6. Amano T, Matsushita A, Hatanaka Y et al: Expression and functional analyses of circadian genes in mouse oocytes and preimplantation embryos: Cry1 is involved in the meiotic process independently of circadian clock regulation. Biol Reprod, 2009; 80: 473–83
7. Namihira M, Honma S, Abe H et al: Circadian rhythms and light responsiveness of mammalian clock gene, Clock and BMAL1, transcripts in the rat retina. Neurosci Lett, 1999; 271: 1–4
8. Mishima K, Tozawa T, Satoh K et al: The 3111T/C polymorphism of hClock is associated with evening preference and delayed sleep timing in a Japanese population sample. Am J Med Genet B Neuropsychiatr Genet, 2005; 133B: 101–4
9. Patke A, Murphy PJ, Onat OE et al: Mutation of the human circadian clock gene CRY1 in familial delayed sleep phase disorder. Cell, 2017; 169: 203–15.e13
10. Tsuchiya Y, Minami Y, Umemura Y et al: Disruption of MeCP2 attenuates circadian rhythm in CRISPR/Cas9-based Rett syndrome model mouse. Genes Cells, 2015; 20: 992–1005
11. Jiang Y: Correlation between occupational stress and sleep quality of field workers in oil fields: Xinjiang Medical University, 2014
12. Hicks RE, Bahr M, Fujiwara D: The occupational stress inventory-revised: Confirmatory factor analysis of the original inter-correlation data set and model. Personality and Individual Differences, 2010; 48: 351–53
13. Li J, Lan YJ, Wang ZM et al: Occupational stress scale (OSI-R) reliability and validity verification. Chinese Journal of Industrial Hygiene And Occupational Diseases, 2001; 19: 190–93
14. Buysse DJ, Reynolds CF 3rd, Monk TH et al: The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. Psychiatry Res, 1989; 28: 193–213
15. Liu XC, Tang MQ: Reliability and validity of the Pittsburgh Sleep Quality Index. Zhong Hua Shen Jing Ke Za Zhi, 1996; 29: 29103–7
16. Yang XW, Liu ZJ, Zhao PQ et al: [A study of the occupational stress norm and it' s application for the technical group and scientific research group]. Wei Sheng Yan Jiu, 2006; 35: 781–84 [in Chinese]
17. Jiang Y: Study on the influence of interaction of environment and genetics on sleep quality of different occupational populations in Xinjiang: Xinjiang Medical University, 2017
18. Silber MH: Sleep disorders. Neurol Clin, 2001; 19: 173–86
19. Chien TW, Hsu SY, Tai C et al: Using Rasch analysis to validate the revised PSQI to assess sleep disorders in Taiwan's hi-tech workers. Community Ment Health J, 2008; 44: 417–25
20. Nomura K, Nakao M, Takeuchi T, Yano E: Associations of insomnia with job strain, control, and support among male Japanese workers. Sleep Med, 2009; 10: 626–29
21. Bunney BG, Bunney WE: Mechanisms of rapid antidepressant effects of sleep deprivation therapy: Clock genes and circadian rhythms. Biol Psychiatry, 2013; 73: 1164–71
22. Utge SJ, Soronen P, Loukola A et al: Systematic analysis of circadian genes in a population-based sample reveals association of TIMELESS with depression and sleep disturbance. PLoS One, 2010; 5: e9259
23. Lou F: Association between CLOCK, PER2, PER3 gene polymorphisms and Parkinson's disease in Han population of northern China: Xinjiang Medical University, 2018
24. Cao YL, Cui QT, Tang CH, Chang X: [Association of CLOCK gene T3111C polymorphism with attention deficit hyperactivity disorder and related sleep disturbances in children]. Zhongguo Dang Dai Er Ke Za Zhi, 2012; 14: 285–88 [in Chinese]
25. Benedetti F, Dallaspezia S, Fulgosi MC et al: Actimetric evidence that CLOCK 3111 T/C SNP influences sleep and activity patterns in patients affected by bipolar depression. Am J Med Genet B Neuropsychiatr Genet, 2007; 144b: 631–35
26. Antypa N, Mandelli L, Nearchou FA et al: The 3111T/C polymorphism interacts with stressful life events to influence patterns of sleep in females. Chronobiol Int, 2012; 29: 891–97
27. Serretti A, Gaspar-Barba E, Calati R et al: 3111T/C clock gene polymorphism is not associated with sleep disturbances in untreated depressed patients. Chronobiol Int, 2010; 27: 265–77
28. Serretti A, Cusin C, Benedetti F et al: Insomnia improvement during antidepressant treatment and CLOCK gene polymorphism. Am J Med Genet B Neuropsychiatr Genet, 2005; 137b: 36–39
29. Hardeland R: Melatonin in aging and disease-multiple consequences of reduced secretion, options and limits of treatment. Aging Dis, 2012; 3: 194–225
30. Tan G, Guo XY, Luo AL: Perioperative melatonin and cortisol levels and postoperative sleep disturbances in elderly patients. Anhui Medical Journal, 2013; 34: 1141–44
31. Wang F: Effect of intranasal glucocorticoid combined with montelukast sodium on sleep status and memory ability of children with mild obstructive sleep apnea syndrome. International Medicine and Health Guidance News, 2016; 22: 2317–19

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]