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Original Article

Correlation between ESR1, BDNF, or APOE polymorphisms and postmenopausal sleep disorder in the Shanghai area

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Abstract: Objective: Postmenopausal sleep disorder is an important health problem that affects women. In this study, we analyzed single nucleotide polymorphisms (SNPs) in the genes encoding estrogen receptor α (ESR1), brain-derived neurotrophic factor (BDNF), and apolipoprotein E (APOE) and investigated the relationship between these SNPs and postmenopausal sleep disorder. Methods: A total of 308 postmenopausal women (mean age: 56.61 ± 3.49 years) completed a questionnaire regarding basic postmenopausal information and were assessed using the Pittsburgh Sleep Quality Index Scale (PSQI). On the basis of the PSQI score, the participants were divided into the normal sleep group (PSQI ≤ 7; n=194) and the sleep disorder group (PSQI > 7; n=114). An improved multiple ligase detection reaction (iMLDR) was performed to analyze the following 12 loci: ESR1: rs1062577, rs2228480, rs2234693, and rs9340799; BDNF: rs16917237, rs4074134, rs4923461, and rs6265; and APOE: rs2075650, rs6857, rs7412, and rs76945. In addition, the haplotypes for these loci were determined. Results: Of the 12 SNP loci, 11 were unrelated to postmenopausal sleep disorder. A significant difference was observed in the ESR1 SNP locus rs2228480 between the normal sleep group and the sleep disorder group ($P < 0.05$). Conclusion: The ESR1 SNP locus rs2228480 is related to postmenopausal sleep disorder.

Keywords: Single nucleotide polymorphism, postmenopausal women, sleep disorder

Introduction

Postmenopausal women experience a series of physiological and psychological changes, and sleep disorder is one of the most common clinical symptoms [1]. Epidemiological surveys have shown that the incidence of postmenopausal sleep disorder is between 35% and 60% [2]. Krystal et al showed that in postmenopausal women, low levels of circulating estrogen are related to a high incidence of insomnia [3]. Mille et al demonstrated that estrogen shortens sleep latency, reduces the frequency of awakening during sleep, and increases total sleep time [4]. Estrogen's physiological role is mediated by the estrogen receptor, which specifically binds estrogen and thereby regulates gene expression. Previous studies have shown that estrogen receptor α (ESR1) is related to sleep [5]. Brain-derived neurotrophic factor (BDNF) is one of the key members of the neurotrophin family. Specifically, BDNF is involved in sleep homeostasis, and its serum level is related to sleep disorders in women [6]. Apolipoprotein E (APOE) is involved in lipid redistribution, maintaining the innate cholesterol balance of the central nervous system. In addition, APOE is involved in neuronal repair and function remodeling, having a direct effect on the growth of synapses. There are different subtypes of APOE, and studies have shown that APOE subtype ε4 allele is related to obstructive sleep-related breathing disorders [7].

Few studies have been conducted locally or internationally to investigate single nucleotide polymorphisms (SNPs) in women with postmenopausal sleep disorder and even fewer have been conducted in Asian populations. We conducted a cross-sectional study on postmenopausal sleep disorder in the Shanghai area of China and investigated the relationship

between 12 SNP loci in the genes encoding ESR1, BNDF, and APOE and postmenopausal sleep disorder. This work was performed to provide a theoretical basis for treatment.

Materials and methods

Subjects

A total of 308 women who visited our hospital medical examination center for a health check-up between January and June 2012 were recruited into this survey. Inclusion criteria: Age 40-65; postmenopausal for ≥ 12 months; no history of sleep disorders, no use of sleeping pills in the past 6 months; no history of glucocorticoid or sex hormone therapy; and voluntary consent to participate. Exclusion criteria: regular menstruation or postmenopausal for ≤ 12 months; a history of sleep disorders or the use of sleeping pills in the past 6 months; estrogen replacement therapy (HRT) or postmenopausal hormone therapy (MHT) in the past 6 months; mental illness or inability to answer the questions in the survey; or ongoing treatment for a malignant tumor. Each subject completed a written questionnaire survey, and a physician then collected the basic information and determined the Pittsburgh Sleep Quality Index Scale (PSQI) score. On the basis of PSQI score, the subjects were divided into the normal sleep group (PSQI ≤ 7; n=194) and the sleep disorder group (PSQI > 7; n=114). This study was approved by the Ethics Committee of our hospital, and each subject signed the written informed consent before the study.

PSQI scale

Sleep quality in the past month was evaluated using the PSQI (Buysse et al [8]). The scale consists of 18 items relating to 7 dimensions of sleep disorders, and the scores of each dimension are added to obtain the total score (range: 0-21). A high total score indicates poor sleep quality [9]. In general, a PSQI score ≥ 8 indicates good sleep quality [10].

Sample collection and DNA extraction

A fasting peripheral venous blood sample (5 mL) was collected in the morning, placed in an EDTA-treated tube, and stored in a -80°C freezer after stepwise cryopreservation (4°C → -20°C → -40°C → -80°C, over at least 24 hours). Genomic DNA was extracted with isopropanol precipitation, standardized to 10 ng/μl, and then quantitatively analyzed with a UV spectrophotometer (criterion of DNA purity: $OD_{260\ nm}/OD_{280\ nm}$=1.6-1.8; DNA concentration (μg/μl)=$OD_{260\ nm}$ × DNA dilution factor × 50/1000). The concentration of the working solution was in the range of 0.1-0.5 μg/μl.

Genotype and SNP selection

DNA was isolated from the genome of peripheral leukocytes, and improved multiple ligase detection reaction (iMLDR) [11] was performed for genotyping using the ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster CA, USA). The SNP loci were selected as follows: 1) functional SNP loci in the exons, 3'UTR, and 5'UTR of relevant genes with a frequency > 10% in Chinese populations were selected from the SNP database of the National Center of Biotechnology Information (NCBI); 2) according to the international human genome haplotype mapping project (HapMap), all of the tag SNPs in the genes encoding ESR1, BDNF, and APOE were identified with r^2 > 0.8 and a minor allele frequency (MAF) > 0.05; 3) a literature review was performed to identify hot SNPs in sleep-related genes that have not been validated in Chinese populations. We ultimately identified the following 12 SNP loci after analyzing functional SNPs, Tag SNPs, and hot SNPs: ESR1: rs1062577, rs2228480, rs2234693, and rs9340799; BDNF: rs16917237, rs4074134, rs4923461, and rs6265; and APOE: rs2075650, rs6857, rs7412, and rs769450.

Statistical analysis

A Hardy-Weinberg equilibrium test was performed to analyze genotype and allele frequency, and the χ^2 test was performed for between-group comparison. SPSS v20.0 software (Chicago, IL, USA) was used for the statistical analyses. Normally distributed measurement data are expressed as the mean ± standard deviation ($x \pm s$), and one-way analysis of variance (ANOVA) was performed for between-group comparison of age, height, weight, education, income, age at menopause, and sleep quality. Moreover, independent samples t-tests were performed for between-group comparison; a non-parametric test was used for non-normally distributed data or data with heterogeneity of variance. $P < 0.05$ was considered statistically significant. Plink analysis software (http//pngu.mgh.harvard.edu/~purcell/plink/) [12] was us-

Table 1. Comparison of the general information

	Postmenopausal Women			P
	N (± SD)	Normal Sleep Group (± SD)	Sleep Disorder Group (± SD)	
n	308	19 (62.99%)	114 (37.01%)	
Age (years)	56.61 ± 3.49	56.61 ± 3.43	56.61 ± 3.60	0.55
45-49	11	7 (63.64)	4 (36.36)	
50-54	58	33 (56.90)	25 (43.10)	
55-59	181	118 (65.19)	63 (34.81)	
60-64	55	35 (63.64)	20 (36.36)	
65-69	3	1 (33.33)	2 (66.67)	
Height (cm)	160.59 ± 5.39	160.84 ± 5.33	160.17 ± 5.48	0.79
Weight (kg)	60.30 ± 8.54	60.70 ± 8.30	59.61 ± 8.92	0.87
Age at initial menstruation (years)	15.04 ± 1.90	15.02 ± 1.86	15.07 ± 1.96	0.17
Age at menopause (years)	50.41 ± 3.13	50.53 ± 3.22	50.21 ± 2.97	0.13
Sleep quality score	7.49 ± 3.49	5.25 ± 1.19	11.31 ± 2.70	0.00*
Occupation				
Employed	75		49 (65.33)	26 (34.67)
Retired	233		145 (62.23)	88 (37.77)
Education				
No formal education	7		4 (57.14)	3 (48.86)
Primary school	16		6 (37.5)	10 (62.5)
Junior high school	49		32 (65.31)	17 (34.69)
Senior high school or technical school	127		72 (56.69)	55 (43.30)
Associate degree	36		25 (69.44)	11 (30.56)
Bachelor degree or above	73		55 (75.34)	18 (24.66)
Personal monthly income (RMB Yuan)				
< 1000	17		7 (41.18)	10 (58.82)
1000-3000	151		94 (62.25)	57 (37.74)
3000-5000	108		70 (64.81)	38 (35.19)
5000-10000	29		20 (68.97)	9 (31.03)
> 10000	3		3 (100)	0 (0)
Marital status				
Married	308		194 (100)	114 (100)
Single or divorced	0		0	0
Smoking				
Non-smoker	306		192 (98.97)	114 (100)
History of smoking or currently smoking	2		2 (1.03)	0
Drinking				
Drinking	302		191 (98.45)	111 (97.37)
Non-drinker	6		3 (1.55)	3 (2.63)
History of drinking or currently drinking				

*P < 0.05, indicates significant difference.

ed for regression analysis of sleep disorder and SNPs. Haploview software [13] was used to determine the genotype, linkage disequilibrium (LD) of the haplotype block, and the LD coefficient r^2. Phase v2.0 software [14] was used to calculate the frequency of each haplotype.

Results

Basic information of subjects

A total of 308 postmenopausal women aged 46 to 65 years (mean: 56.61 ± 3.49) were

Table 2. Genotype and allele frequencys

SNP name	Allele variants	SNP in dbSNP	Gene	Genotype frequencies (N, %)			Allele frequencies (N, %)	
SNP 1	A/T	rs1062577	ESRα	AA (22, 7.1)	AT (128, 41.6)	TT (158, 51.3)	A (172, 27.9)	T (444, 72.1)
SNP 2	G/A	rs2228480	ESRα	GG (186, 60.4)	GA (103, 33.4)	AA (19, 6.2)	G (475, 77.1)	A (141, 22.9)
SNP 3	C/T	rs2234693	ESRα	CC (53, 17.2)	CT (139, 45.1)	TT (116, 37.7)	C (245, 39.8)	T (371, 60.2)
SNP 4	G/A	rs9340799	ESRα	GG (11, 3.6)	GA (104, 33.8)	AA (193, 62.6)	G (126, 20.5)	A (490, 79.5)
SNP 5	G/T	rs1691723	BDNF	GG (93, 30.2)	GT (149, 48.4)	TT (66, 21.4)	G (335, 54.4)	T (281, 45.6)
SNP 6	C/T	rs4074134	BDNF	CC (99, 32.1)	CT (158, 51.3)	TT (51, 16.6)	C (356, 57.8)	T (260, 42.2)
SNP 7	A/G	rs4923461	BDNF	AA (101, 32.8)	AG (154, 50.0)	GG (53, 17.2)	A (356, 57.8)	G (260, 42.2)
SNP 8	C/T	rs6265	BDNF	CC (91, 29.5)	CT (155, 50.3)	TT (62, 20.1)	C (337, 54.7)	T (279, 45.3)
SNP 9	A/G	rs2075650	APOE	AA (253, 82.1)	AG (51, 16.6)	GG (4, 1.3)	A (557, 90.4)	G (59, 9.6)
SNP10	C/T	rs6857	APOE	CC (254, 82.5)	CT (50, 16.2)	TT (4, 1.3)	C (558, 90.6)	T (58, 9.4)
SNP 11	C/T	rs7412	APOE	CC (266, 86.4)	CT (40, 13.0)	TT (2, 0.6)	C (572, 92.9)	T (44, 7.1)
SNP 12	A/G	rs769450	APOE	AA (14, 4.5)	AG (100, 32.5)	GG (194, 63.0)	A (128, 20.8)	G (488, 79.2)

Table 3. General analysis of SNPs

SNP name	Allele variants	SNP in dbSNP	Gene	Location	HWE (P)	MAF in HCB	MAF in this study
SNP 1	A/T	rs1062577	ESR1	3'-UTR	0.569	0.27	0.279
SNP 2	G/A	rs2228480	ESR1	cds-synon	0.355	0.20	0.229
SNP 3	C/T	rs2234693	ESR1	intron	0.308	0.41	0.398
SNP 4	G/A	rs9340799	ESR1	intron	0.509	0.23	0.205
SNP 5	G/T	rs16917237	BDNF	intron	0.661	0.49	0.456
SNP 6	C/T	rs4074134	BDNF	intron	0.366	0.44	0.422
SNP 7	A/G	rs4923461	BDNF	intron	0.662	0.44	0.422
SNP 8	C/T	rs6265	BDNF	None	0.786	0.48	0.453
SNP 9	A/G	rs2075650	APOE	intron	0.440	0.11	0.096
SNP10	C/T	rs6857	APOE	3'-UTR	0.340	0.11	0.094
SNP 11	C/T	rs7412	APOE	missense	0.712	0.09	0.071
SNP 12	A/G	rs769450	APOE	intron	0.808	0.19	0.208

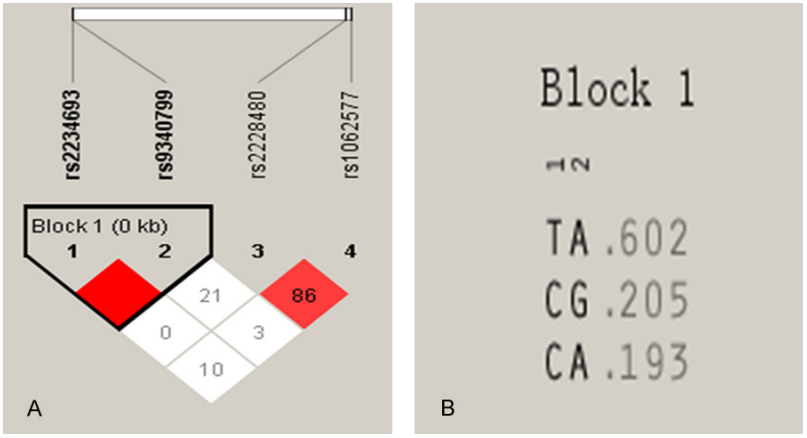

Figure 1. Linkage disequilibrium pattern in the ESR1 gene (A). The red area indicates strong linkage disequilibrium. The maximum D' value was 1. (B) PHASE software was used to analyze the haplotype block, and the results revealed three haplotypes: TA (60.2%), CG (20.5%), and CA (19.3%), with a frequency of > 1% for all three haplotypes.

recruited into this study. Of these women, 194 (62.99%) were included in the normal sleep group and 114 (37.01%) in the sleep disorder group. The PSQI score was significantly higher in the sleep disorder group than in the normal sleep group (11.31 ± 2.70 vs 5.25 ± 1.19, P=0.55). No significant between-group difference was observed in age, height, weight, or other demographic data (P > 0.05) (**Table 1**).

Allele, genotype frequency, Hardy-Weinberg equilibrium test, and linkage disequilibrium

The genotype of the SNP loci and the allele frequency of the ESR1, BDNF and APOE genes are shown in **Table 2**. The MAF was > 0.01 for all SNPs, and the genotype frequency of these SNPs was consistent with the Hardy-Weinberg equilibrium (P > 0.05, **Table 3**). The linkage disequilibrium values of the alleles of these three genes are shown in **Figures**

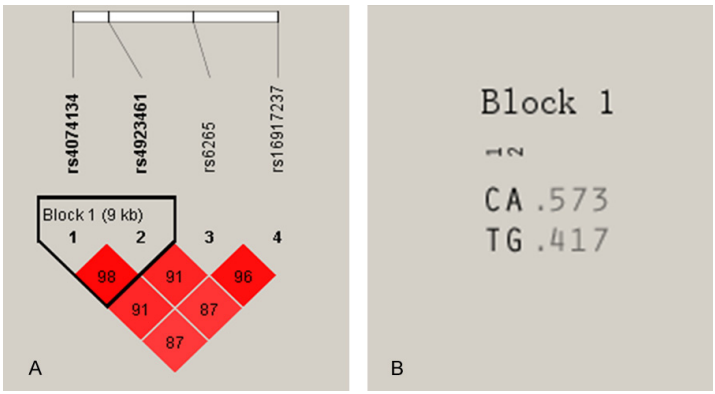

Figure 2. Linkage disequilibrium pattern in the BDNF gene (A). The red area indicates strong linkage disequilibrium. The maximum D' value was 0.98. (B) PHASE software was used to analyze the haplotype block, and the results showed two haplotypes: CA (57.3%) and TG (41.7%), with a frequency of > 1% for both haplotypes.

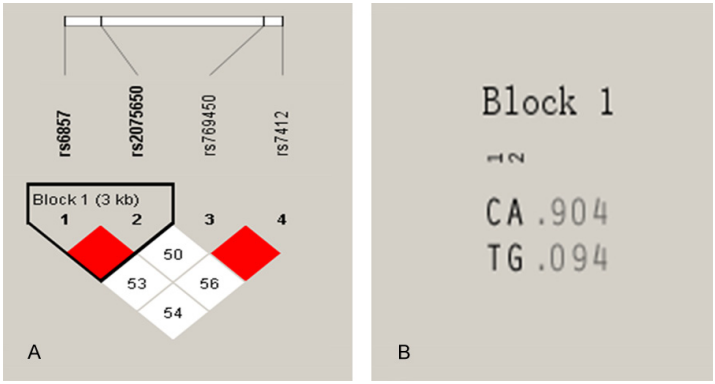

Figure 3. Linkage disequilibrium pattern in the APOE gene (A). The red area indicates strong linkage disequilibrium. The maximum D' value was 1. (B) PHASE software was used to analyze haplotype block, and the results showed two haplotypes: CA (90.4%) and TG (9.4%), with a frequency of > 1% for both haplotypes.

1A, **2A** and **3A**. In the ESR1 gene, rs2234693 and rs9340799 form a block (maximum D'=1.0) with three haplotypes (**Figure 1B**). In the BDNF gene, rs4074134 and rs4923461 form a block (maximum D'=0.98) with two haplotypes (**Figure 2B**). In the APOE gene, rs2075650 and rs6857 form a block (maximum D'=1) with two haplotypes (**Figure 3B**).

Relationship between the SNP loci and postmenopausal sleep disorder

The results of correlation analysis of the 12 SNP loci and the existence of sleep disorder in the two groups are shown in **Tables 4**-**6**. One-way ANOVA showed no significant correlations between the SNP loci and the scores for any of the seven factors or the total sleep score. After controlling for the covariates of age, height, education, income, marital status, smoking, and drinking, logistic regression analysis of genotype and sleep quality showed that genotype AA of rs2075650 and genotype AG of rs2228480 were risk factors for sleep disorder (**Table 7**). Further logistic regression analysis showed no correlation between haplotype and sleep disorder (**Table 8**).

Relationship between SNP polymorphisms or haplotype and postmenopausal sleep disorder

Linear regression analysis was performed to analyze the relationship between each locus and its haplotype and sleep quality. After controlling for the covariates of age, education, and income, the chi-square test showed a significant difference in the genotype frequency of ESR1 rs2228480 ($P < 0.05$). No significant difference in the genotype frequency of the other SNP loci was observed between the two groups ($P > 0.05$) (**Table 9**).

Discussion

Postmenopausal sleep disorder is one of the most common clinical symptoms in postmenopausal women [15] and is related to postmenopausal status, race, and geological area. A review of 24 Chinese and foreign surveys of postmenopausal sleep disorder in 63,542 women (40-55 years of age) from different countries showed that Caucasian women are more vulnerable to postmenopausal sleep disorder, followed by Asians, whereas Hispanic women are the least vulnerable [16, 17]. In addition, studies have shown that postmenopausal sleep disorder is related to the interaction between genetic and environmental factors, the heritability of sleep disorders being approximately 57% [18]. To date, however, few studies have been conducted to investigate the relationship between postmenopausal sleep disorder and specific SNPs.

Table 4. Correlation analysis of ESR1 SNP loci and sleep

Sleep Quality	rs1062577			P	rs2228480			P	rs2234693			P	*rs9340799*			P
	AA	AT	TT		AA	GA	GG		CC	CT	TT		GG	GA	AA	
PSQI score	7.05 ± 3.39	7.52 ± 3.57	7.53 ± 3.45	0.44	7.63 ± 3.42	8.04 ± 3.78	7.17 ± 3.30	0.13	6.75 ± 2.81	7.83 ± 3.65	7.42 ± 3.54	0.16	6.91 ± 3.70	7.63 ± 3.58	7.45 ± 3.44	0.77
Sleep quality	1.09 ± 0.43	1.23 ± 0.51	1.24 ± 0.54	0.72	1.21 ± 0.42	1.26 ± 0.61	1.20 ± 0.47	0.66	1.11 ± 0.47	1.24 ± 0.51	1.25 ± 0.54	0.23	1.09 ± 0.54	1.22 ± 0.52	1.23 ± 0.51	0.67
Time to fall asleep	0.86 ± 1.17	1.06 ± 1.09	1.06 ± 1.09	0.84	1.05 ± 1.31	1.17 ± 1.16	0.92 ± 1.05	0.18	0.94 ± 0.91	1.11 ± 1.22	0.94 ± 1.05	0.42	0.91 ± 1.04	1.08 ± 1.16	0.99 ± 1.10	0.77
Sleep time	1.45 ± 0.86	1.44 ± 0.93	1.44 ± 0.93	0.79	1.47 ± 0.77	1.52 ± 1.00	1.34 ± 0.89	0.24	1.21 ± 0.82	1.48 ± 0.90	1.41 ± 0.97	0.18	1.27 ± 0.79	1.38 ± 0.92	1.44 ± 0.92	0.76
Sleep efficiency	1.32 ± 1.17	1.30 ± 1.03	1.30 ± 1.03	0.55	1.47 ± 0.84	1.48 ± 1.10	1.25 ± 0.97	0.17	1.09 ± 0.90	1.43 ± 1.01	1.34 ± 1.05	0.12	0.82 ± 0.75	1.44 ± 0.96	1.32 ± 1.05	0.13
Sleep disorder	1.05 ± 0.21	1.12 ± 0.37	1.12 ± 0.37	0.60	1.05 ± 0.23	1.13 ± 0.36	1.12 ± 0.36	0.69	1.13 ± 0.39	1.11 ± 0.33	1.13 ± 0.36	0.86	1.18 ± 0.60	1.13 ± 0.33	1.11 ± 0.35	0.82
Sleeping pills	0	0.07 ± 0.36	0.07 ± 0.36	0.95	0.11 ± 0.46	0.10 ± 0.45	0.06 ± 0.33	0.68	0.06 ± 0.31	0.11 ± 0.48	0.04 ± 0.28	0.38	0.27 ± 0.65	0.11 ± 0.48	0.05 ± 0.29	0.10
Functional disorder during the day	1.27 ± 0.55	1.30 ± 0.54	1.30 ± 0.54	0.83	1.26 ± 1.56	1.38 ± 0.51	1.27 ± 0.52	0.22	1.21 ± 0.57	1.35 ± 0.52	1.30 ± 0.05	0.26	1.36 ± 0.67	1.29 ± 0.53	1.31 ± 0.51	0.88

Table 5. Correlation analysis of BDNF SNP loci and sleep

Sleep Quality	rs16917237			P	rs4074134			P	rs4923461			P	rs6265			P
	GG	GT	TT		CC	P	TT		AA	AG	GG		CC	CT	TT	
PSQI score	7.25 ± 3.60	7.52 ± 3.40	7.77 ± 3.60	0.64	7.19 ± 3.60	7.57 ± 3.49	7.82 ± 3.30	0.53	7.30 ± 3.67	7.52 ± 3.46	7.49 ± 3.50	0.72	7.25 ± 3.62	7.50 ± 3.34	7.82 ± 3.68	0.61
Sleep quality	1.20 ± 0.52	1.21 ± 0.50	1.29 ± 0.55	0.53	1.21 ± 0.48	1.21 ± 0.53	1.29 ± 0.54	0.57	1.23 ± 0.51	0.52 ± 0.04	0.53 ± 0.07	0.61	1.21 ± 0.53	1.20 ± 0.49	1.31 ± 0.56	0.37
Time to fall asleep	1.00 ± 1.10	1.05 ± 1.12	0.95 ± 1.10	0.82	0.97 ± 1.12	1.09 ± 1.13	0.88 ± 1.03	0.45	1.00 ± 1.13	1.08 ± 1.12	0.85 ± 1.03	0.41	0.99 ± 1.11	1.06 ± 1.11	0.94 ± 1.11	0.71
Sleep time	1.30 ± 0.96	1.42 ± 0.86	1.55 ± 0.95	0.25	1.30 ± 0.97	1.41 ± 0.88	1.63 ± 0.89	0.12	1.33 ± 0.98	1.39 ± 0.87	1.62 ± 0.88	0.15	1.32 ± 0.97	1.39 ± 0.87	1.60 ± 0.93	0.17
Sleep efficiency	1.31 ± 1.03	1.34 ± 1.00	1.39 ± 1.02	0.88	1.28 ± 1.02	1.35 ± 1.00	1.41 ± 1.04	0.74	1.31 ± 1.03	1.34 ± 1.00	1.42 ± 1.03	0.82	1.31 ± 1.04	1.34 ± 0.98	1.39 ± 1.06	0.89
Sleep disorder	1.08 ± 0.34	1.13 ± 0.34	1.17 ± 0.41	0.26	1.08 ± 0.34	1.13 ± 0.33	1.18 ± 0.43	0.28	1.09 ± 0.35	1.12 ± 0.33	1.17 ± 0.43	0.40	1.08 ± 0.34	1.12 ± 0.33	1.18 ± 0.43	0.23
Sleeping pills	0.05 ± 0.31	0.07 ± 0.40	0.11 ± 0.43	0.70	0.05 ± 0.30	0.08 ± 0.42	0.10 ± 0.41	0.73	0.05 ± 0.30	0.08 ± 0.43	0.09 ± 0.41	0.72	0.05 ± 0.31	0.07 ± 0.40	0.11 ± 0.45	0.65
Functional disorder during the day	1.30 ± 0.55	1.30 ± 0.53	1.32 ± 0.47	0.97	1.29 ± 0.56	1.30 ± 0.51	1.33 ± 0.47	0.90	1.30 ± 0.56	1.30 ± 0.51	1.43 ± 0.48	0.87	1.30 ± 0.55	1.31 ± 0.53	1.31 ± 0.47	0.98

Table 6. Correlation analysis of APOE SNP loci and sleep

Sleep Quality	rs2075650			P	rs6857			P	rs7412			P	rs769450			P
	AA	AG	GG		CC	CT	TT		CC	CT	TT		GG	GA	AA	
PSQI score	7.36 ± 3.42	8.08 ± 3.89	8.25 ± 2.07	0.37	7.37 ± 3.41	8.06 ± 3.93	8.25 ± 2.06	0.40	7.50 ± 3.54	7.33 ± 3.06	7.50 ± 6.36	0.69	7.55 ± 3.58	7.41 ± 3.28	7.29 ± 3.91	0.93
Sleep quality	1.21 ± 0.51	0.27 ± 0.53	1.25 ± 0.50	0.74	1.21 ± 0.51	1.28 ± 0.54	1.25 ± 0.50	0.70	1.23 ± 0.53	1.15 ± 0.43	1.50 ± 0.11	0.48	1.20 ± 0.50	1.26 ± 0.53	1.36 ± 0.63	0.37
Time to fall asleep	0.908 ± 1.10	1.20 ± 1.18	0.75 ± 0.50	0.41	0.99 ± 1.10	1.18 ± 1.19	2.00 ± 0.82	0.47	1.03 ± 1.11	0.93 ± 1.05	1.50 ± 2.12	0.72	1.06 ± 1.13	0.95 ± 1.07	0.86 ± 1.10	0.62
Sleep time	1.35 ± 0.90	1.47 ± 1.09	2.00 ± 0.82	0.3	1.39 ± 0.89	1.46 ± 1.01	1.50 ± 1.00	0.38	1.40 ± 0.92	1.43 ± 0.90	2.50 ± 0.71	0.24	1.41 ± 0.93	1.39 ± 0.90	1.50 ± 0.94	0.91
Sleep efficiency	1.30 ± 0.98	1.53 ± 1.14	1.50 ± 1.00	0.32	1.30 ± 0.98	1.52 ± 1.15	1.50 ± 0.58	0.36	1.33 ± 1.03	1.43 ± 0.87	1.50 ± 2.12	0.83	1.32 ± 0.98	1.39 ± 1.05	1.21 ± 1.12	0.78
Sleep disorder	1.11 ± 0.35	1.14 ± 0.35	1.50 ± 0.58	0.08	1.11 ± 0.35	1.14 ± 0.35	0	0.08	1.12 ± 0.35	1.13 ± 0.40	1.00 ± 0.00	0.89	1.14 ± 0.38	1.09 ± 0.32	1.07 ± 0.27	0.46
Sleeping pills	0.08 ± 0.40	0.06 ± 0.31	0	0.87	0.08 ± 0.40	0.06 ± 0.31	0	0.88	0.09 ± 0.41	0	0	0.40	0.10 ± 0.44	2.02 ± 0.2	0.14 ± 0.54	0.20
Functional disorder during the day	1.28 ± 0.50	1.41 ± 0.61	1.25 ± 0.50	0.28	1.28 ± 0.50	1.42 ± 0.61	1.25 ± 0.50	0.23	1.31 ± 0.52	1.28 ± 0.55	1.50 ± 0.71	0.81	1.31 ± 0.53	1.31 ± 0.51	1.14 ± 0.54	0.49

Table 7. Relationship between SNP genotype and sleep disorder

Variable	B	SE	Significance Level	OR	OR (95% CI)	
					Lower Limit	Upper Limit
Age	0.014	0.042	0.734	1.014	0.934	1.101
Height (cm)	0.008	0.028	0.758	1.009	0.956	1.064
Weight (kg)	0.012	0.016	0.447	1.013	0.981	1.046
Education	0.273	0.145	0.059	1.314	0.990	1.745
Occupation	-0.015	0.344	0.964	0.985	0.502	1.932
Marital status	0[b]					
Income	-0.052	0.231	0.822	0.949	0.604	1.492
[rs1062577=A/A]	0.181	0.554	0.744	1.198	0.404	3.548
[rs1062577=A/T]	-0.300	0.286	0.293	0.740	0.423	1.296
[rs1062577=T/T]	0[b]					
[rs16917237=G/G]	1.003	1.463	0.493	2.727	0.155	48.001
[rs16917237=G/T]	0.222	0.799	0.781	1.249	0.261	5.980
[rs16917237=T/T]	0[b]					
[rs2075650=A/A]	15.323	1.076	0.000	4514498.016	548034.149	37188726.987
[rs2075650=G/A]	0.350	1.112	0.753	1.419	0.161	12.542
[rs2075650=G/G]	0[b]					
[rs2228480=A/A]	-0.183	0.572	0.749	0.832	0.271	2.554
[rs2228480=G/A]	-0.592	0.287	0.039	0.553	0.315	0.972
[rs2228480=G/G]	0[b]					
[rs2234693=C/C]	0.471	0.492	0.339	1.601	0.610	4.201
[rs2234693=C/T]	0.018	0.334	0.958	1.018	0.529	1.959
[rs2234693=T/T]	0[b]					
[rs4074134=C/C]	1.708	2.081	0.412	5.517	0.093	325.630
[rs4074134=T/C]	0.289	1.566	0.853	1.335	0.062	28.724
[rs4074134=T/T]	0[b]					
[rs4923461=A/A]	-1.064	2.097	0.612	0.345	0.006	21.022
[rs4923461=G/A]	0.083	1.538	0.957	1.086	0.053	22.154
[rs4923461=G/G]	0[b]					
[rs6265=C/C]	-0.977	1.642	0.552	0.376	0.015	9.401
[rs6265=C/T]	-0.326	0.947	0.730	0.722	0.113	4.615
[rs6265=T/T]	0[b]					
[rs6857=C/C]	-14.630	0.000		4.430E-07	4.430E-07	4.430E-07
[rs6857=C/T]	0[b]					
[rs6857=T/T]	0[b]					
[rs7412=C/C]	0.732	1.509	0.628	2.079	0.108	39.983
[rs7412=C/T]	0.709	1.541	0.646	2.031	0.099	41.679
[rs7412=T/T]	0[b]					
[rs769450=A/A]	-0.029	0.614	0.962	0.971	0.291	3.234
[rs769450=A/G]	-0.086	0.279	0.758	0.918	0.532	1.584
[rs769450=G/G]	0[b]					
[rs9340799=A/A]	0.525	0.811	0.518	1.690	0.345	8.282
[rs9340799=G/A]	0.348	0.771	0.651	1.417	0.313	6.416
[rs9340799=G/G]	0[b]					

b. This parameter is set as zero because of its redundancy.

For the first time, we report on the relationship between postmenopausal sleep disorder and SNPs in the genes encoding ESR1, BDNF, and APOE in Chinese women. The results showed a

Table 8. Relationship between haplotype and sleep disorder

Variable[a]	B	SE	Significance Level	OR	OR (95% CI)	
					Lower Limit	Upper Limit
Age	0.016	0.041	0.695	1.016	0.938	1.101
Height (cm)	0.004	0.027	0.879	1.004	0.953	1.058
Weight (kg)	0.018	0.016	0.265	1.018	0.987	1.050
Education	0.258	0.139	0.064	1.294	0.985	1.700
Occupation	0.028	0.334	0.934	1.028	0.534	1.978
Marital status	0[b]					
Income	0.025	0.226	0.914	1.025	0.658	1.597
[rs2234693=C/C]	0.410	0.482	0.394	1.507	0.586	3.875
[rs2234693=C/T]	-0.077	0.322	0.811	0.926	0.492	1.741
[rs2234693=T/T]	0[b]					
[rs4074134=C/C]	1.572	1.915	0.412	4.814	0.113	205.264
[rs4074134=T/C]	-0.142	1.470	0.923	0.867	0.049	15.479
[rs4074134=T/T]	0[b]					
[rs4923461=A/A]	-0.964	1.876	0.607	0.381	0.010	15.058
[rs4923461=G/A]	0.372	1.449	0.797	1.451	0.085	24.815
[rs4923461=G/G]	0[b]					
[rs6857=C/C]	0.845	1.043	0.418	2.328	0.302	17.971
[rs6857=C/T]	0.639	1.075	0.552	1.895	0.231	15.583
[rs6857=T/T]	0[b]					
[rs769450=A/A]	-0.009	0.598	0.989	0.992	0.307	3.200
[rs769450=A/G]	-0.111	0.266	0.677	0.895	0.531	1.508
[rs769450=G/G]	0[b]					
[rs9340799=A/A]	0.447	0.789	0.571	1.564	0.333	7.342
[rs9340799=G/A]	0.388	0.751	0.605	1.475	0.338	6.426
[rs9340799=G/G]	0[b]					

a. Reference category. b. This parameter is set as zero because of its redundancy.

significant difference in the genotype frequency of ERS1 rs2228480 between the sleep disorder group and the normal sleep group among postmenopausal women. Currently, most postmenopausal studies on ESR1 polymorphisms focus on age at menopause, postmenopausal osteoporosis, and female breast cancer; few studies have focused on the role of ESR1 in postmenopausal sleep disorder.

Eichling et al showed that postmenopausal sleep disorder is related to low estrogen levels [19]. In postmenopausal women, physiological changes include ovarian failure [20], decreased estrogen production, and low circulating estrogen levels. Estrogen receptors are distributed in various tissues and organs, such as the uterus, brain, bone tissue, nervous system, and blood vessels. Moreover, estrogen is involved regulating various brain functions, such as learning, memory, and cognition [21]. Hence, low estrogen levels and estrogen receptor mutations can affect various brain functions. In addition, studies have shown that vasomotor symptoms are important causes of postmenopausal sleep disorder [22], and estrogen receptors are also widely distributed in the vascular system. The ESR1 gene encodes an estrogen receptor, which binds estrogen and mediates its effects. This study showed that an ESR1 polymorphism was related to postmenopausal sleep disorder. Different ESR1 alleles likely exhibit differential binding of estrogen, thereby affecting the function of this hormone and potentially resulting in postmenopausal sleep disorder. This study also showed a significant difference in the frequency of the rs2228480 genotype GA between the sleep disorder and normal sleep groups (42.1% vs 28.4%, $P < 0.05$). Thus, rs2228480 is likely one of the genetic factors that affects the risk for postmenopausal sleep disorder. A Japanese study also showed that the A/G polymorphism rs2228480 is related to postmenopausal hormone therapy [23]. Moreover, for middle-aged women, sleep disorder is closely related to depression and cognitive disorders, and other studies [24] have demonstrated that ESR1 gene plays a role in sleep disorders.

BDNF gene plays an important role in neurotransmission and both maintaining and reinforcing the delayed stage of long-term potentiation and learning and memory [25]. In this study, we selected four BDNF SNPs (rs16917237, rs4074134, rs4923461, rs6265), and the results showed that the frequency of each SNP was consistent with that found in Asian populations using the Hapmap database and with the Hardy-Weinberg equilibrium. However,

Table 9. Relationship between SNP polymorphism or haplotype and sleep disorder

Gene	SNP	Genotype	Normal Sleep Group (n, %)	Sleep Disorder Group (n, %)	χ^2	P
ESR1	rs1062577	AA	6 (5.3)	16 (8.2)		
		AT	50 (40.3)	78 (40.2)	1.132	0.568
		TT	58 (50.9)	100 (51.5)		
	rs2228480	GG	126 (64.9)	60 (52.6)		
		GA	55 (28.4)	48 (42.1)	6.107	0.047*
		AA	13 (6.7)	6 (5.3)		
	rs2234693	CC	37 (19.1)	16 (14.0)		
		CT	84 (43.3)	55 (48.2)	1.448	0.485
		TT	73 (37.6)	43 (37.7)		
	rs9340799	GG	7 (3.6)	4 (3.5)		
		GA	66 (34.0)	38 (33.3)	0.019	0.990
		AA	121 (62.4)	72 (63.2)		
BDNF	rs16917237	GG	62 (32.0)	31 (27.2)		
		GT	93 (47.9)	56 (49.1)	0.991	0.609
		TT	39 (20.1)	27 (23.7)		
	rs4074134	CC	68 (35.1)	31 (27.1)		
		CT	96 (49.5)	62 (54.4)	2.095	0.351
		TT	30 (15.5)	21 (18.4)		
	rs4923461	AA	68 (35.1)	33 (28.9)		
		AG	95 (49.0)	59 (51.8)	1.387	0.500
		GG	31 (16.0)	22 (19.3)		
	rs6265	CC	60 (30.9)	31 (27.2)		
		CT	97 (50.0)	58 (50.9)	0.641	0.726
		TT	37 (19.1)	25 (21.9)		
APOE	rs2075650	AA	162 (83.5)	9 (29.8)		
		AG	30 (15.5)	21 (18.4)	0.787	0.675
		GG	2 (1.0)	2 (1.8)		
	rs6857	CC	162 (83.5)	92 (80.7)		
		CT	30 (15.5)	20 (17.5)	0.549	0.760
		TT	2 (1.0)	2 (1.8)		
	rs7412	CC	168 (86.6)	98 (86.0)		
		CT	25 (12.9)	15 (13.2)	0.152	0.927
		TT	1 (0.5)	1 (0.9)		
	rs769450	AA	9 (4.6)	5 (4.4)		
		AG	61 (31.4)	39 (34.2)	0.252	0.882
		GG	124 (63.9)	70 (61.4)		

*P < 0.05, indicates significant difference.

this study showed no correlation between the four BDNF SNP loci and two haplotypes and postmenopausal sleep disorder. To date, few studies have been conducted to investigate the role of APOE in sleep quality. Some studies showed that the APOE ε4 allele is related to obstructive sleep-related breathing disorders [26]. This study showed no significant difference between the two groups in terms of the genotype frequencies of the four examined APOE SNP loci and the two haplotypes.

In summary, the ESR1 SNP rs2228480 is related to postmenopausal sleep disorder, but studies with larger sample sizes are necessary to confirm the findings of this study.

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Disclosure of conflict of interest

None.

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