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Association Between CACNA1C Polymorphisms and Insomnia in a Chinese Han Population: A Case-Control Population-Based Analysis

Liucui Chen
University of South China
Huaxin Li
University of South China
Haijun Yuan
Foresea Life Insurance Guangxi Hospital
Wei Gao
University of South China
Feng Liu
University of South China
Bing Ma
University of South China
Yingmei Ding
University of South China
Xiaofen Liu
University of South China
Mei Yuan (meimeihaijun@163.com)
University of South China https://orcid.org/0000-0002-2110-6168

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Abstract

A limited number of genome-wide association studies (GWAS) have shown that *CACNA1C* is a potential candidate gene for insomnia. The present study sought to investigate the association of *CACNA1C* gene polymorphisms and insomnia in a Chinese Han population. Twenty-one single nucleotide polymorphisms (SNPs) in the *CACNA1C* were genotyped by using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) with a Sequenom MassARRAY system in 205 patients with insomnia and 154 healthy controls. Data from all participants were systematically collected. Association of the polymorphisms with insomnia was evaluated by statistical analysis. Linkage disequilibrium (LD) and haplotype analysis were performed with Haploview v4.2 software. After adjustment for multiple comparisons and gender, age, body mass index (BMI), hypertension and glycated hemoglobin (HbA1c), only the rs2302729 in the recessive model (adjusted odds ratio [AOR]= 0.414, 95%CI= 0.220-0.771, *P*= 0.004) related to insomnia achieved significance by the false discovery rate (FDR) Benjamini and Hochberg (BH) criterion. The haplotypes rs2302729-rs1051375, CA and TG were significantly associated with insomnia (*P*<0.05). Our findings contributed important evidence for the confirming of *CACNA1C* as a susceptibility gene for insomnia in the samples of Chinese Han population.

Introduction

Insomnia is one of the most common sleep disorders encountered in the population, frequently characterized by the subjective complaint of difficulty falling or maintaining sleep, or non-restorative sleep, producing significant daytime symptoms, including difficulty concentrating and mood disturbances. Studies from Western populations show that the incidence of insomnia was 6.8% to 19.2% (Jansson-Fröjmark and Linton 2008; Pallesen et al. 2014; Ford et al. 2015). While a Chinese cross-sectional study recruited 2982 aged participants indicated that the prevalence of occasional insomnia and frequent insomnia was up to 17.07% and 12.17% respectively (Zhuang et al. 2016). These studies indicated that insomnia is a widespread public health problem globally, especially in China. Insomnia is associated with several negative sequela, including fatigue, impaired memory, inattention, irritability, and depression. Epidemiological and longitudinal studies have also shown that insomnia is a risk factor for the development of a variety of health problems, such as new-onset mood, anxiety and depression, impaired immune function, diabetes, obesity and cognitive impairment, and death related to cardiovascular disease (Bollu and Kaur 2019; Fernandez-Mendoza and Vgontzas 2013).

Despite insomnia being widespread and severely affected, the exact cause of insomnia is still largely unknown, and a better understanding of its pathogenic mechanism is essential to improve the quality of life and clinical outcomes of insomnia. Previous studies also have shown that non-genetic factors such as older age, female, depression, poor perceived health, pain, obesity, and socioeconomic status may increase the risk of insomnia (Hartz et al. 2007). Most notably, several aspects of sleep behavior such as timing, duration and quality have been demonstrated to be heritable (Byrne et al. 2013). The identification of the potential susceptibility genes and gene loci, and the exploration of the molecular genetics of insomnia are required to facilitate the early diagnosis and prevention of it, and personalized treatment.

However, the contribution of genetics to its etiology and pathophysiology remains largely unknown, while initial studies point in a few directions. Several genome wide association studies (GWAS) have been conducted on sleep phenotypes in humans (Byrne et al. 2013; Scheinfeldt et al. 2015; Lane et al. 2017; Hammerschlag et al. 2017). A series of recent studies demonstrated that insomnia can be attributed to many circadian clock genetic factors, such as *TSHZ2*, *ABCC9*, *FBXO15*, *SORCS1*, *ELOVL2* (Scheinfeldt et al. 2015), *CRY1* (Patke et al. 2017) and *CLOCK* (Semenova et al. 2017). Additionally, a twin modeling study (Lind et al. 2015) also indicated insomnia symptoms were found to be modestly heritable. Nevertheless, many of these analyses had small sample sizes, or based on certain ethnic, and even many associations were not replicated in other independent samples (Byrne et al. 2013; Parsons et al. 2013).

The voltage-dependent calcium channel, L-type, alpha 1C subunit (*CACNA1C*) gene encodes CACNA1c, whose function is to mediate the influx of calcium ions into the cell upon membrane depolarization, and is mainly involved in neurotransmitter release, neuronal excitability (Xu et al. 2015), and gene regulation (Bhat et al. 2012). Recently, studies have shown that *CACNA1C* gene, a previously reported bipolar disorder associated gene, is one of the most interesting candidate genes related to sleep disorders. A set of single nucleotide polymorphisms (SNPs) in *CACNA1C* gene are associated with sleep quality and sleep latency, two insomnia characteristics, in the Australian and British populations. (Byrne et al. 2013; Parsons et al. 2013), supporting its pivotal role as a notable candidate gene for insomnia. While, those findings were not replicated by other GWAS (Scheinfeldt et al. 2015; Lane et al. 2017; Hammerschlag et al. 2017). In addition, GWAS also has many limitations (Emily 2018): First, tag-SNPs, only a selection of SNPs, were genotyped by SNPs array but not all SNPs. Second, GWAS suffer from a multiple testing issue which might be different from one statistical test to another due to the large number of SNPs tested when considering significance at the genome level. Finally, covariates which can play an important role in the analysis of disease susceptibility, while GWAS didn't taken it into consideration.

Given the susceptibilities of *CACNA1C* polymorphisms in insomnia is still unknown in Chinese population who had a high incidence of insomnia, we undertook an evaluation of 21 candidate SNPs in *CACNA1C* within an independent sample of Chinese Han population in this report.

Material And Methods

Study Population and DNA Extraction

A case-control study included 205 patients with chronic insomnia and 154 age-matched healthy volunteers from the Department of Neurology at the Second Affiliated Hospital, University of South China (Hengyang, China) between Jul 2016 and Dec 2018. All subjects were unrelated Han Chinese from Hunan province. The diagnosis of chronic insomnia is based on the patient's subjective complaints about poor sleep quality, difficulty starting or maintaining sleep, or early to wake, along with reports of severe distress or daytime consequences, and duration of at least 3 or more nights per week for three months or longer, according to the diagnostic criteria from the International Classification of Sleep Disorder, 3rd (ICSD-3) (American Academy of Sleep Medicine 2014). It is well-established that chronic insomnia disorder in

ICSD-3 can be combined with many other medical conditions, such as the dependence of these comorbidities on pain, mental disorders, life events, and pregnancy (Bollu and Kaur 2019; Fernandez-Mendoza and Vgontzas 2013; Nacar and Taşhan 2019). As these conditions were not appropriate for insomnia genetic studies. Therefore, subjects with these conditions should exclude from the study. Specifically, the exclusion criteria are: 1) patients with a standard total Self-rating Depression Scale (SDS) score of >62 points, or a standard total Self-rating Anxiety Scale (SAS) score of >59 points; 2) subjects with pregnancy, chronic pain, malignant tumor, dementia, stroke, moderate to severe obstructive sleep apnea hypopnea syndrome, chronic obstructive pulmonary disease, congestive heart failure, asthma, cirrhosis, renal impairment, and moderate to severe mental disorders; 3) subjects with a history of major emotional trauma six months before sampling, or a history of taking corticosteroids or psychotropic drugs.

All patients, whose chief complaint was poor sleeping quality, received the final diagnosis by the patient's clinical manifestations, clinical history, Pittsburgh Sleep Quality Index (PSQI), SAS, SDS and Polysomnography (PSG) results. The scoring used for PSQI, SDS, and SAS was conducted according to reference (Zung 1965; Zung 1971; Tsai et al. 2005). As described in the study (Tsai et al. 2005), a total PSQI score >7 points indicates sleep disturbance, and the higher scores indicate more serious the condition.

The study protocol was approved by the Human Ethics Committees Review Board at the University of South China (no. 201602001), and signed informed consent was obtained from all participants. Data from all participants were systematically collected, including gender, age, body weight index (BMI), glycated hemoglobin (HbA1c), and blood pressure. History of insomnia were collected from the patients.

Venous blood samples were collected from all participants. Every genomic DNA was extracted from the blood samples using TIANamp Genomic DNA Kit (TIANGEN BIOTECH, Beijing, China). The DNA concentration was determined with the NanoDrop 2000 (Thermo Fisher Scientific, MA, USA), and this was followed by dilution to a final concentration of 500ng/uL. Diluted extracts and extracted serum samples were stored at -20°C until further analysis.

Candidate SNPs Selection and Primer Designing

Candidate SNPs in the *CACNA1C* were selected from several large genome-wide association studies (GWAS) (Byrne et al. 2013; Parsons et al. 2013) and the NCBI database (http://www.ncbi.nlm.nih.gov/projects/SNP/), and each SNP must have a minimum allele frequency (MAF) of greater than 0.05 in the Chinese Han population. A total of 25 tagSNPs were initially selected, such as rs2051990, rs7301906, rs7304986, rs7316184, rs16929275, rs16929276 and rs16929278 in intron 3; rs2302729 in intron 9; rs215976, rs216008, rs1051375 and rs1544514 in exon; rs2041135, rs2470433, rs4765975, rs7302540, rs7316246, rs7957163, rs10466907, rs10713809, rs11062319, rs11353034, rs11429670, rs12809807 and rs35440646 located in 3'UTR. Because the regions of homologous or near-homologous sequence in the genome can compete with the target sequence of the genetic locus for primer hybridization that leads to PCR failure, rs16929278 in intron 3, and rs10713809,

rs11429670 and rs35440646 in 3'UTR were not tested. Finally, twenty-one SNPs were chosen as candidates for the following experiments. Primers for detection potential polymorphisms were designed with MassARRAY Assay Designer v3.1 (Sequenom, CA, USA). Pairs of PCR primers and single base extension (SBE) primers were provided for each SNP, and listed in **Tables 1** and **2**.

Genotype

Genotyping of each SNP locus was performed using a Sequenom MassARRAY system using matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) (Sequenom, CA, USA) at BGI (Beijing, China). All experimental operations were carried out in accordance with the manufacturers' instructions. Each total 5μL PCR reaction contained 0.625μL 1.25×PCR Buffer, 1.000 μL (500nM each) PCR primer mix (synthesized by Thermo Fisher Scientific, MA, USA), 0.325μL 25mM $MgCl_2$, 0.1 μL 25 mM dNTP mix, 0.1000 μL HotStar Taq (5U/μL), and 1 μL of DNA sample (20ng), and 1.850 μL HPLC water. Primary PCR was performed on the GeneAMP PCR system 9700 (Thermo Fisher Scientific, MA, USA) with the following conditions: at 94°C for 5 min, 45 cycles of 94°C for 20 s, 56°C for 30 s, and 72°C for 60 s, and a final extension at 72°C for 3 min. Then, non-incorporated dNTPs in the PCR products were dephosphorylated by adding a cocktail of 1.53 μL HPLC water, 0.17 μL Shrimp Alkaline Phosphatase (SAP) Buffer and 0.3μL SAP enzyme (1U/μL) (Sequenom, CA, USA) incubated on the GeneAMP PCR system 9700 at 37°C for 20 min, and then followed by 85°C for 5 min. SBE reaction contained 7 μL of SAP-treated PCR products and 2μL iPLEX mix (Sequenom, CA, USA). The iPLEX mix contained 0.2000μL iPLEX buffer, 0.2000μL iPLEX Termination mix, 0.940μL extend primer mix(0.625μM:1.25μM), 0.041μL iPLEX enzyme and 0.619μL HPLC water. The SBE reaction was performed with the following conditions: 94 °C for 30 s, followed by 40 cycles of one step at 94°C for 5 s with five subcycles of 52 °C for 5 s and 80 °C for 5s, and followed by 72°C for 3 min. A total of 16 μL molecular HPLC water and each extension product were added to CLEAN Resin 384/6 MG Dimple plate (Sequenom, CA, USA) for further purification. Samples were rotated for 30 min on a tube rotator. After treatment, approximately 10 nL of products were spotted onto a 384-well SpectroCHIP bioarray (Sequenom, CA, USA), with the MassARRAY Nanodispenser RS1000 (Sequenom, CA, USA). The time of flight of these ionized products depends on the mass of each allele that will be measured by the mass spectrometer. The results were visualized on the MassArray Typer Analyzer v4.0 (Sequenom, CA, USA).

Statistical analysis

Clinical data and gene frequencies were analyzed using the *t*-test, Mann-Whitney U test and the χ^2 test with SPSS 22.0 (IBM, NY, USA). Values were presented as mean ± SD, median (M) and range, or the number and percentage (%) based on the type of data. We calculated original genotype frequencies for each of the 21 polymorphisms in insomnia and control groups, and assessed Hardy-Weinberg equilibrium for each SNP locus among controls. Considering the imbalance of the possible clinical insomnia risk factors between the case group and the control group, and the errors in multiple comparisons, the SNPs which were significant differences in the distributions (*P*-value <0.05 in the initial results) of genotype frequencies between insomnia and control participants were selected for further analysis. To identify the

effect of genetic models on insomnia, analyses were performed using dominant (wild-type homozygote versus heterozygote and mutant-homozygote), recessive (wild-type homozygote and heterozygote versus mutant-homozygote), and additive (wild-type homozygote versus mutant-homozygote) modes for each polymorphism (Zhu et al. 2019). We estimated the genetic odds ratio (OR) using logistic regression analyses to compute relative risks and 95% confidence intervals (CI). To address the use of a pooled control group, genetic analyses were adjusted for the matching factors of gender, age, BMI, hypertension, and HbA1c. We then perform false discovery rate (FDR) Benjamini and Hochberg procedure (BH) (Benjamini et al. 2001), a type of significance-level adjustment for controlling the error in multiple comparisons, to account for the increased probability of reporting false positive results through multiple tests. The procedure for controlling the FDR at level 0.05, and FDR (BH) thresholds for each of the 21 individual SNPs was calculated as described (Benjamini et al. 2001). Linkage disequilibrium (LD) and haplotype analysis were performed with Haploview v4.2 software, and a detailed description of the haplotype analysis is given elsewhere (Gabriel et al. 2002). A difference of P <0.05 (two-sided) indicates significant.

Results

Clinical data

The clinical characteristics of the subjects in the study are shown in **Table 3**. There were significant differences between the two groups in the systolic blood pressure (SBP) and HbA1c levels between the two groups (P <0.05). However, there was no significant difference in the age, gender, BMI, and diastolic blood pressure (DBP) between the case and control groups (P >0.05). The results of PSQI evaluation showed that all insomnia patients with a total score >7 points, and the overall PSQI average score was (14.23 ± 2.85), which suggests the cases had severe insomnia disorder. The measurement results of the SAS and SDS scale showed that none of the 205 patients had moderate to severe depression or anxiety. Furthermore, the PSG records revealed no evidence of moderate to severe obstructive sleep apnea hypopnea syndrome, or other sleep disorder.

Genotype Association Analysis

Table 4 lists the 21 polymorphisms examined and the observed genotype frequencies at each gene locus among the subjects. All the SNP markers were accorded with the Hardy-Weinberg equilibrium (HWE) (P>0.05). As shown, there were significant differences in the distributions of genotype frequencies only in three loci, rs1051375 and rs216008 in exon, and rs2302729 in intron 9, between the insomnia group and the control group (P<0.05). These three loci were selected for further analysis.

Genetic Analysis

Genetic contributions of the SNP with alleles to insomnia were analyzed by using dominant, recessive, and additive models' assessment. **Table 5** presents three selected SNPs which, in any model of inheritance, were found in the initial analysis. As shown, following our multivariate logistic regressions

and risk factor-adjusted analyses, only the recessive model and additive model of the rs216008, rs2302729 and rs1051375 polymorphism were found to have a risk-adjusted *P*-value less than 0.05 for association with insomnia. However, in analyses using the multiple tests with the FDR (BH) criterion, only the rs2302729 significant in the FDR analysis remained in the recessive model (AOR =0.416, 95%CI =0.220-0.781, *P* =0.004). In comparison with the CC+CT genotype at rs2302729, the TT genotype decreased the risk of the disease by 0.416 times, suggesting that the major C allele may be a risk factor for insomnia.

Haplotype association and Linkage disequilibrium

Haplotype structures of the *CACNA1C* gene were analyzed. Haplotype association analysis suggested twenty-one SNPs were identified that detected two LD blocks, among which four haplotypes were identified, as shown in **Figure 1** and **Table 6**. The results showed that block 1 consisted of two closely linked SNPs (rs2302729 and rs1051375) with |D'| value is 0.83, and block 2 included five linked SNPs (rs7316264, rs12809807, rs10466907, rs4765975, and rs7957163) with |D'| value is 0.81. In particular, the block 1, rs2302729-rs1051375, CA and TG haplotypes were associated with the risk of insomnia in the Chinese Han population (*P*<0.05), while the rs7316264-rs12809807-rs10466907-rs4765975-rs7957163 haplotypes were not associated with insomnia in this population (*P*>0.05, **Table 6**).

Discussion

In this case-control study, of the 21 SNPs analyzed here, we found only the recessive model of the rs2302729 in *CACNA1C* gene to be strongly associated with insomnia in our Chinese Hunan Han population, after adjustment for risk factor-adjusted analyses and multiple comparisons. We also observed a significant association between rs2302729-rs1051375, CA and TG haplotypes with insomnia.

The *CACNA1C* gene, located on chromosome 12p13.3, encodes an α-1 subunit of the L-type voltage-dependent gated calcium channel (CACNA1c), which consists of 24 transmembrane segments, forming the pore mediate calcium ions (Ca^{2+}), a second messenger in the phosphatidylinositol system, pass into the cell. The channel transiently increases the membrane permeability for calcium-mediated cell membrane depolarization, playing an essential role in neurotransmitter release, neuronal excitability, and gene regulation (Mortensen 2013; Nakao et al. 2015). Sleep-wake cycle is regulated by several neurotransmitters, including calcium ions, and neuronal excitability (Xu et al. 2015). It is worth noting that the CACNA1c may be involved in mental illnesses, such as bipolar disorder and major depressive disorder (Kabir et al. 2017), and insomnia is the most common manifestation of those mental illness. The effect of CACNA1c on insomnia may overlap with bipolar disorder. Moreover, treating sleep regulation center with voltage-gated L-type Ca^{2+} channel (LTCC) blocker diltiazem can significantly increase pentobarbital-induced total sleep (TS), non-rapid eye movement (NREM) sleep and slow wave sleep (SWS) ratio in NREM sleep (Cui et al. 2011). Therefore, CACNA1c may participate in insomnia by regulating calcium influx, altering nerve excitability and neurotransmitter release. In recent years, as a candidate gene contributing to essential insomnia, *CACNA1C* has been highlighted in corresponding

studies, despite the lack of replication (Byrne et al. 2013; Parsons et al. 2013; Kantojärvi et al. 2017). From a clinical and statistical research perspective, our data provide preliminary evidence for the establishment of *CACNA1C* as a susceptibility gene for insomnia.

In this study, only the recessive model of rs2302729 was found to have a risk-adjusted *P*-value less than FDR (BH) thresholds (**Table 5**). The rs2302729 located in intron 9 of *CACNA1C*. Current studies suggest that introns can alter not only transcript levels by affecting the rate of transcription, transcript stability, and nuclear export, but also the efficiency of precursor messenger RNA (pre-mRNA) splicing and mRNA translation (Shaul 2017). Sequence variants, such as SNPs, may lead to disorders in gene transcription, splicing and translation, resulting in abnormalities in gene expression and function (Caminsky et al. 2014). As reported, different splicing products of voltage-controlled calcium channels could lead to different functional effects, which can affect the influx of extracellular calcium ions and the activation of calcium signaling (Kim et al. 2005), and the subsequent activation of mitogen-activated protein kinase (MAPK) signal in the suprachiasmatic nuclei, and then stimulate potential downstream transcription factors to regulate the expression of biological clock genes (Obrietan et al. 1998) leading to insomnia. Several variations in *CACNA1C* introns have been reported to predispose carriers to the development of insomnia (Byrne et al. 2013; Parsons et al. 2013; Kantojärvi et al. 2017). Though current research has failed to replicate the same SNP results, we successfully found an association of rs2302729 in intron 9 in recessive model with insomnia, and found that the rs2302729 T allele had a decreased risk of insomnia. It is unclear whether and how this mutation alters the expression of *CACNA1C*. It's worth noting that, by analyzing the nucleotide sequence, we found that the C allele at the rs2302729 locus could create a CpG site, which may serve as methylation site and regulate the gene expression, just like the rs1051375 locus (Kim et al. 2016).

Though the recessive model and additive model of the rs216008 and rs1051375 polymorphism were found to have a risk-adjusted *P*-value less than 0.05 for association with insomnia in the present study, the associations were no longer significant by using the multiple tests with the FDR (BH) criterion (Table 5). This suggested that the rs216008 and rs1051375 polymorphisms may still be potential SNPs for insomnia. In fact, the alterations in the rs1051375 and rs216008 SNPs don't directly affect the primary structure of the CACNA1c. Nevertheless, previous study showed that genetic associations at rs1051375 has been observed in the Korean population of patients with bipolar disorder, and the G allele, the minor allele in the rs1051375 SNP, creates a CpG site containing a cytosine nucleotide adjacent to a guanine nucleotide (Kim et al. 2016). Most importantly, 70-80% of cytosines in the mammalian genome CpG sites are methylated (Jabbari and Bernardi, 2004), and DNA methylation pattern of *CACNA1C* is specific in neuronal cells (Nishioka et al. 2013). It is worth mentioning that the bipolar disorder is characterized by circadian rhythm disturbances. In the current study, we excluded patients with moderate to severe mental disorders, though it is unclear whether insomnia patients with rs1051375 are predisposed to anxiety and depression. Interestingly, we find that the rs2302729 and rs1051375 constituted a closely linkage disequilibrium block (Figure 1 and Table 6), and the rs2302729-rs1051375, CA and TG haplotypes were also significantly associated with insomnia in our population ($P<0.05$). Given that the recessive model of rs2302729 polymorphism is highly associated with insomnia, it was shown here that the

polymorphism in rs1051375 was related to the insomnia before adjustment using FDR correction, probably due to the two SNPs were in significantly linkage disequilibrium. While another study of interindividual variability in mRNA expression and 12 splicing loci of *CACNA1C* in 65 heart tissue samples indicate that the mRNA ratio of *CACNA1C* for the rs216008 C and T alleles did not differ significantly from that of DNA ratio in all samples tested (Wang et al. 2006). However, the effect of rs216008 and rs1051375 on the expression of *CACNA1C* is still unclear. Therefore, additional research is needed to determine whether and how the mutations alter the expression and function of the *CACNA1C* gene in insomnia.

Our study also has some limitations. First, compared to the GWAS study, the sample size in our report was relatively small. Second, For the analysis of the genetic factors of insomnia, cases with many comorbidities on pain, mental disorders, life events, and many other confounding conditions were excluded from the study, and the selected polymorphisms were then entered into the risk factor-adjusted analysis which further controlled for gender, age, BMI, hypertension, and diabetes. While there were relatively few risk factors included in the logistic regression analysis. At last, since our controls, who were healthy individuals, had no complaints of sleep disorders and emotion disorders, they had not undergone SAS, SDS, and PSG assessments, and the comparison of these indexes between the insomnia group and the controls was not performed.

Conclusions

In summary, this study analyzed the relationship between twenty-one candidate polymorphisms in the *CACNA1C* gene and insomnia. Our results indicate that rs2302729 polymorphism in *CACNA1C* gene is significantly associated with insomnia, and *CACNA1C* may be a susceptibility gene for insomnia in the samples of Chinese Han population. Further well-designed studies with a larger population sample are still warranted to validate our findings, and additional investigations also are necessary to define the mechanism underlying this relationship.

Declarations

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The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. The authors would like to thank to the participants for taking part in the study.

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Conflicts of interest The authors declare that they have no conflict of interest.

Availability of data and material The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Authors' contributions The study designed by Mei Yuan. Material preparation, data collection and analysis were performed by Liucui Chen, Haijun Yuan and Wei Gao. The first draft of the manuscript was written by Liucui Chen, Huaxin Li, Bing Ma and Yingmei Ding. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval The study protocol was approved by the Human Ethics Committees Review Board at the University of South China (no. 201602001).

Consent to participate Signed informed consent was obtained from all participants.

Consent for publication Not applicable.

References

1. American Academy of Sleep Medicine (2014) International Classification of Sleep Disorders. 3rd ed. Darien IL.
2. Benjamini Y, Drai D, Elmer G et al (2001) Controlling the false discovery rate in behavior genetics research. Behav Brain Res 125: 279-284. https://doi.org/10.1016/s0166-4328(01)00297-2
3. Bhat S, Dao D T, Terrillion C E et al (2012) CACNA1C (Cav1.2) in the pathophysiology of psychiatric disease. Progress in neurobiology 99:1-14. https://doi.org/10.1016/j.pneurobio.2012.06.001
4. Bollu P C, Kaur H (2019) Sleep Medicine: Insomnia and Sleep. Missouri medicine 116:68-75.
5. Byrne E M, Gehrman P R, Medland S E et al (2013) A genome-wide association study of sleep habits and insomnia. Am J Med Genet B Neuropsychiatr Genet 162B:439–451. https://doi.org/10.1002/ajmg.b.32168
6. Caminsky N, Mucaki EJ, Rogan PK (2014) Interpretation of mRNA splicing mutations in genetic disease, review of the literature, guidelines for information-theoretical analysis. F1000Res 3:282. https://doi.org/10.12688/f1000research.5654.1
7. Cui S Y, Cui X Y, Zhang J et al (2011) Ca^{2+} modulation in dorsal raphe plays an important role in NREM and REM sleep regulation during pentobarbital hypnosis. Brain research 1403:12–18. https://doi.org/10.1016/j.brainres.2011.05.064
8. Emily M (2018) Power comparison of Cochran-Armitage trend test against allelic and genotypic tests in large-scale case-control genetic association studies. Stat Methods Med Res. 27:2657-2673. https://doi.10.1177/0962280216683979
9. Fernandez-Mendoza J, Vgontzas AN (2013) Insomnia and its impact on physical and mental health. Curr Psychiatry Rep 15:418. https://doi.org/10.1007/s11920-013-0418-8

10. Ford E S, Cunningham T J, Giles W H et al (2015) Trends in insomnia and excessive daytime sleepiness among U.S. adults from 2002 to 2012. Sleep Med 16:372–378. https://doi.org/10.1016/j.sleep.2014.12.008
11. Gabriel S B, Schaffner S F, Nguyen H et al (2002) The structure of haplotype blocks in the human genome. Science (New York, N.Y.) 296:2225–2229. https://doi.org/10.1126/science.1069424
12. Hammerschlag A R, Stringer S, de Leeuw C A et al (2017) Genome-wide association analysis of insomnia complaints identifies risk genes and genetic overlap with psychiatric and metabolic traits. Nature genetics 49:1584–1592. https://doi.org/10.1038/ng.3888
13. Hartz A J, Daly J M, Kohatsu N D et al (2007). Risk factors for insomnia in a rural population. Annals of epidemiology 17:940–947. https://doi.org/10.1016/j.annepidem.2007.07.097
14. Jabbari K, Bernardi G (2004) Cytosine methylation and CpG, TpG (CpA) and TpA frequencies. Gene 333:143-149. https://doi.org/10.1016/j.gene.2004.02.043
15. Jansson-Fröjmark M, Linton SJ (2008) The course of insomnia over one year: a longitudinal study in the general population in Sweden. Sleep 31: 881–886. https://doi.org/10.1093/sleep/31.6.881
16. Kabir Z D, Lee A S, Burgdorf C E et al (2017) Cacna1c in the Prefrontal Cortex Regulates Depression-Related Behaviors via REDD1. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology 42:2032–2042. https://doi.org/10.1038/npp.2016.271
17. Kantojärvi K, Liuhanen J, Saarenpää-Heikkilä O et al (2017) Variants in calcium voltage-gated channel subunit Alpha1 C-gene (CACNA1C) are associated with sleep latency in infants. PloS one 12: e0180652. https://doi.org/10.1371/journal.pone.0180652
18. Kim D Y, Choi H J, Kim J S et al (2005) Voltage-gated calcium channels play crucial roles in the glutamate-induced phase shifts of the rat suprachiasmatic circadian clock. The European journal of neuroscience 21:1215–1222. https://doi.org/10.1111/j.1460-9568.2005.03950.x
19. Kim S, Cho C H, Geum D et al (2016) Association of CACNA1C Variants with Bipolar Disorder in the Korean Population. Psychiatry Investig 13: 453–457. https://doi.org/10.4306/pi.2016.13.4.453
20. Lane J M, Liang J, Vlasac I et al (2017) Genome-wide association analyses of sleep disturbance traits identify new loci and highlight shared genetics with neuropsychiatric and metabolic traits. Nature genetics 49:274–281. https://doi.org/10.1038/ng.3749
21. Lind M J, Aggen S H, Kirkpatrick R M et al (2015) A Longitudinal Twin Study of Insomnia Symptoms in Adults. Sleep 38:1423–1430. https://doi.org/10.5665/sleep.4982
22. Mortensen OV (2013) MKP3 eliminates depolarization-dependent neurotransmitter release through downregulation of L-type calcium channel Cav1.2 expression. Cell Calcium 53:224-230. https://doi.org/10.1016/j.ceca.2012.12.004
23. Nacar G, Taşhan ST (2019) Relationship between sleep characteristics and depressive symptoms in last trimester of pregnancy. Afr Health Sci 19:2934-2944. https://doi.org/10.4314/ahs.v19i4.14
24. Nakao A, Miki T, Shoji H et al (2015). Comprehensive behavioral analysis of voltage-gated calcium channel beta-anchoring and -regulatory protein knockout mice. Frontiers in behavioral neuroscience 9:141. https://doi.org/10.3389/fnbeh.2015.00141

25. Nishioka M, Shimada T, Bundo M et al (2013) Neuronal cell-type specific DNA methylation patterns of the Cacna1c gene. International journal of developmental neuroscience: the official journal of the International Society for Developmental Neuroscience 31:89–95. https://doi.org/10.1016/j.ijdevneu.2012.11.007
26. Obrietan K, Impey S, Storm DR (1998) Light and circadian rhythmicity regulate MAP kinase activation in the suprachiasmatic nuclei. Nat Neurosci 1:693-700. https://doi.org/10.1038/3695
27. Pallesen S, Sivertsen B, Nordhus I H et al (2014) A 10-year trend of insomnia prevalence in the adult Norwegian population. Sleep Med 15:173-179. https://doi.org/10.1016/j.sleep.2013.10.009
28. Parsons M J, Lester K J, Barclay N L et al (2013) Replication of Genome-Wide Association Studies (GWAS) loci for sleep in the British G1219 cohort. American journal of medical genetics. Part B, Neuropsychiatric genetics: the official publication of the International Society of Psychiatric Genetics 162B: 431–438. https://doi.org/10.1002/ajmg.b.32106
29. Patke A, Murphy P J, Onat O E et al (2017) Mutation of the Human Circadian Clock Gene CRY1 in Familial Delayed Sleep Phase Disorder. Cell 169:203–215. https://doi.org/10.1016/j.cell.2017.03.027
30. Scheinfeldt L B, Gharani N, Kasper R S et al (2015) Using the Coriell Personalized Medicine Collaborative Data to conduct a genome-wide association study of sleep duration. American journal of medical genetics. Part B, Neuropsychiatric genetics: the official publication of the International Society of Psychiatric Genetics 168:697–705. https://doi.org/10.1002/ajmg.b.32362
31. Semenova N V, Madaeva I M, Bairova T I et al (2017). 3111T/C Clock Gene Polymorphism in Women with Insomnia. Bulletin of experimental biology and medicine 163:461–464. https://doi.org/10.1007/s10517-017-3828-5
32. Shaul O (2017) How introns enhance gene expression. The international journal of biochemistry & cell biology 91:145–155. https://doi.org/10.1016/j.biocel.2017.06.016
33. Tsai P S, Wang S Y, Wang M Y et al (2005) Psychometric evaluation of the Chinese version of the Pittsburgh Sleep Quality Index (CPSQI) in primary insomnia and control subjects. Quality of life research: an international journal of quality of life aspects of treatment, care and rehabilitation 14: 1943–1952. https://doi.org/10.1007/s11136-005-4346-x
34. Wang D, Papp A C, Binkley P F et al (2006) Highly variable mRNA expression and splicing of L-type voltage-dependent calcium channel alpha subunit 1C in human heart tissues. Pharmacogenetics and genomics 16:735–745. https://doi.org/10.1097/01.fpc.0000230119.34205.8a
35. Xu M, Chung S, Zhang S et al (2015) Basal forebrain circuit for sleep-wake control. Nature neuroscience 18:1641–1647. https://doi.org/10.1038/nn.4143
36. Zhu B, Gao M, Zhang L et al (2019). Association of PTTG1 polymorphism rs1895320, rs2910200 and rs6882742 with non-functioning pituitary adenomas in Chinese Han population: a case-control study. Metabolic brain disease 34:841–846. https://doi.org/10.1007/s11011-018-0364-6
37. Zhuang J, Zhan Y, Zhang F et al (2016) Self-reported insomnia and coronary heart disease in the elderly. Clinical and experimental hypertension (New York, N.Y: 1993) 38:51–55.

https://doi.org/10.3109/10641963.2015.1060983
38. Zung W W (1965) A SELF-RATING DEPRESSION SCALE. Archives of general psychiatry 12: 63–70. https://doi.org/10.1001/archpsyc.1965.01720310065008
39. Zung W W (1971) A rating instrument for anxiety disorders. Psychosomatics 12:371–379. https://doi.org/10.1016/S0033-3182(71)71479-0

Tables

Due to technical limitations, table 1-6 is only available as a download in the Supplemental Files section.

Figures

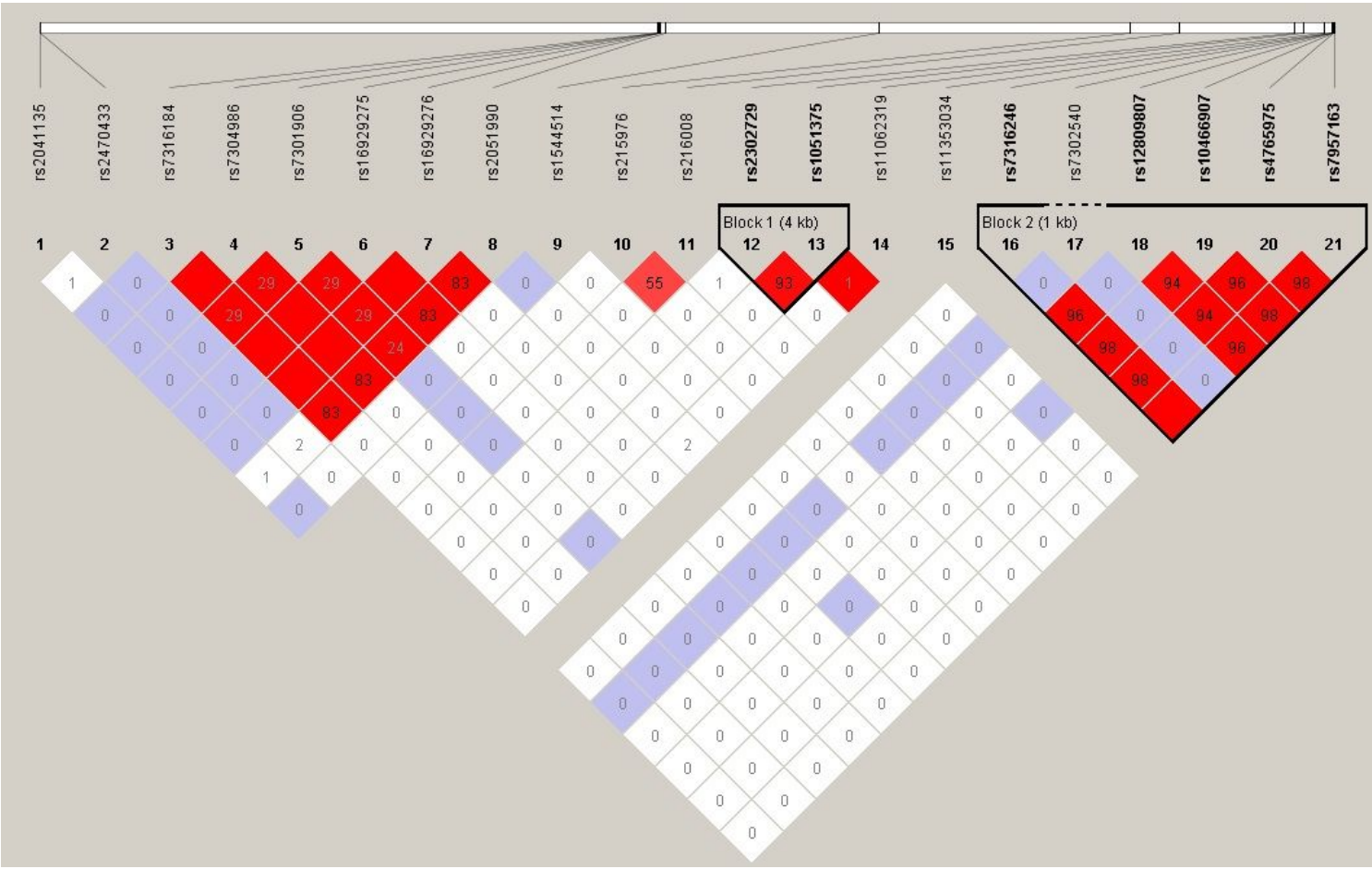

Figure 1

Diagram of the haplotypes and linkage disequilibrium (LD) of twenty-one SNP loci of the CACNA1C gene in Chinese Han subjects. The SNPs and orientation in the CACNA1C gene are shown at the top of the figure. The darker color indicates a greater linkage disequilibrium (LD), and the lighter color indicates a lower LD. Haplotype construction and LD analysis were performed with Haploview v4.2 software.

Supplementary Files

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