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Article

Sleep quality and *OPRM1* polymorphisms: a cross-sectional study among opioid-naive individuals

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Opioidergic system involves in regulation of sleep and wakefulness. It is possible, therefore, that genetic polymorphisms in *OPRM1* influence sleep quality. This study investigated the association of *OPRM1* polymorphisms with subjective sleep quality among opioid-naive individuals. This cross-sectional observational study involved 161 opioid-naive males (mean age = 27.74 years; range: 18−63 years). Subjective sleep quality was assessed with the translated and validated Malay version of the Pittsburgh Sleep Quality Index (PSQI). DNA was extracted from whole blood and subjected to polymerase chain reaction (PCR)-genotyping for two *OPRM1* polymorphisms (118A>G and IVS2+691G>C). Subjects with combined *118A* and *IVS2+691G* alleles (AC haplotype) had significantly lower PSQI scores [mean (SD) = 4.29 (1.76)] compared to those without the haplotype [4.99 (2.50)] (p = 0.004). On the other hand, subjects with combined heterozygous genotype (GC/AG diplotype) had significantly higher PSQI scores compared to those without the diplotype [6.04 (2.48) *vs* 4.54 (2.22), p = 0.004]. In opioid-naive individuals, AC haplotype and GC/AG diplotype for the 118A>G and IVS2+691G>C polymorphisms of *OPRM1* are associated with better and poorer sleep quality, respectively.

Keywords: Sleep-Wake Transition Disorders. Sleep quality. Sleep/genetic. Polymorphism. Pittsburgh Sleep Quality Index. OPRM1. Opioid receptors /μ analysis.

INTRODUCTION

Poor sleep quality has been reported in general population. In German, 36% of the general population slept badly (Hinz *et al.*, 2017). A survey conducted in China reported that the overall prevalence of insomnia was 26.6% among 26,851 subjects from the general population (Tang *et al.*, 2017). A study among 794 medical students in Malaysia found that 16.1% reported bad sleep quality (Zailinawati *et al.*, 2009). In another study, a higher prevalence of poor sleep quality (32.9%) was reported among 1,118 Malaysian tertiary students (Lai, Say, 2013). Shift work, sleep disorders, socioeconomic status, aging, anxiety and the number of chronic disease, amongst others, were found to be significantly associated with poor sleep quality (Guo *et al.*, 2013; Patel *et al.*, 2010; Luo *et al.*, 2013; Hinz *et al.*, 2017; Tang *et al.*, 2017). Poor sleep quality is often associated with accidents (Garbarino *et al.*, 2001; Powell *et al.*, 2007), and an increased in total healthcare and lost productivity costs (Sarsour *et al.*, 2011). In addition, sleep problems are associated with a decreased in health related quality of life (Schubert *et al.*, 2002; Rashid *et al.*, 2012) and have been reported as a risk factor for all-cause mortality (Marshall *et al.*, 2008). More recently, a community-based population study has shown that moderate-to-severe sleep apnea is independently associated with a large increased risk of all-cause mortality, incident stroke, and cancer incidence and mortality (Marshall *et al.*, 2014).

Genetic and environmental factors have been implicated in sleep and sleep problems (Heath *et al.*, 1990; Lessov-Schlaggar *et al.*, 2008; Watson *et al.*, 2006; Genderson *et al.*, 2013). Research suggests that genetic

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differences accounted for at least 33% of the variance in sleep quality and sleep disturbance and 40% of the variance in sleep pattern (Heath *et al.*, 1990). Recently, a twin research has highlighted the importance of genes on the Pittsburgh Sleep Quality Index (PSQI) and the seven components of the PSQI (Genderson *et al.*, 2013). Genetic association studies have provided some progress in understanding the genetic control of sleep and heritability of sleep disorders (Varvarigou *et al.*, 2011; Evans *et al.*, 2013; Palagini, Biber, Riemann, 2014; Wisor, Kilduff, 2005; Millstein *et al.*, 2011; Andretic, Franken, Tafti, 2008). Individual differences in regulation of sleep and wakefulness can be explained by variation within specific genes, and single nucleotide polymorphisms (SNPs) or combinations of SNP alleles that tend to be inherited together (haplotypes) may be associated with sleep in healthy populations and among sleep-disordered patients (Andretic, Franken, Tafti, 2008).

The endogenous opioid peptide β-endorphin is known to be involved in the regulation of sleep and wakefulness (King *et al.*, 1981). Pharmacological studies have shown that the naturally-occurring opioid peptide, β-endorphin, interacts preferentially with opioid receptor, mu 1 (OPRM1). A previous study has shown that β-endorphin and morphine injected intraventricularly produced insomnia in cats (King *et al.*, 1981). Their findings showed that deep slow wave sleep (SWS) was sharply inhibited, rapid eye movement (REM) sleep was entirely suppressed, and light slow wave sleep, occurring in brief, isolated episodes, became the most abundant stage of sleep. They also found that an opioid receptor antagonist, naloxone, injected subcutaneously reversed the effects of β-endorphin and morphine on the two stages of SWS, but it did not counteract the REM-suppressant effect of either β-endorphin or morphine (King *et al.*, 1981). This data may indicate an involvement of an inner opioid in the regulation of sleep-wake cycle (King *et al.*, 1981), and further research suggest the existence of somnogenic effect of opioidergic system in the caudal nucleus tractus solitarius (NTS) (Reinoso-Barbero, Andrés, 1995).

Later, electroacupuncture (EA) of Anmian acupoints has been shown to increase non-rapid eye movement (NREM) sleep, but not REM sleep, during the dark period in rats (Cheng *et al.*, 2011). This data suggest that stimulation of the opiodergic neurons to increase the concentrations of β-endorphin and the involvement of the μ-opioid receptors may also be a mechanism by which acupuncture affects sleep (Cheng *et al.*, 2011). It is possible, therefore, some of the *OPRM1* polymorphisms that affect the density and function, and consequently the signaling efficacy of μ-opioid receptors may contribute to inter-individual variations in the sleep quality. The most frequently studied polymorphism of *OPRM1,* 118A>G (dbSNP rs1799971, Asn40Asp) polymorphism, is found in exon 1. It may affect the μ-opioid receptor N-glycosylation and reduced stability of the receptor (Huang *et al.*, 2012). IVS2+691G>C (dbSNP rs2075572) polymorphism is found within intron 2 at 691 bp downstream of exon 2 (Xin, Wang, 2002; Hoehe *et al.*, 2000; Lötsch, Geisslinger, 2006). It may change the regulation of the expression of OPRM1 gene and may also cause formation of different isoforms of human μ-opioid receptor (Hoehe *et al.*, 2000; Wendel, Hoehe, 1998). Unfortunately, the association between *OPRM1* polymorphisms and sleep quality is unknown and requires exploration. Examining the influence of genetic polymorphisms on sleep quality will be informative in terms of gaining a deeper understanding of sleep and sleep problems. In this study, we aimed to investigate the influence of *OPRM1* polymorphisms on sleep quality among opioid-naive individuals.

MATERIAL AND METHODS

Participants

One hundred and sixty one healthy volunteers who had taken part in a pharmacogenetics study conducted by the Institute for Research in Molecular Medicine (INFORMM), USM, Kota Bharu, Kelantan, and who satisfied inclusion and exclusion criteria were recruited from the local community between March and October 2013. Opioid-naive individual was defined as individual who have not taken any opioids including morphine and methadone to the best of their knowledge and have had two consecutive negative urine screenings results for illicit drugs. Inclusion criteria were: 1) Malay male aged more than 18 years; 2) Free of acute medical, surgical and psychiatric illness; and 3) Free of regular use of alcohol. The exclusion criteria were: 1) individuals who were currently taking illicit benzodiazepines, cannabinoids and barbiturates; 2) individuals on regular anticonvulsants, neuroleptics or analgesics; 3) individuals with chronic or ongoing acute pain; 4) individuals with a history of analgesics ingestion within three days before the study; and 5) individuals with severe cognitive impairment which may interfere with sleep assessments and/or communication.

Written informed consent was obtained from each subject after a complete description of the study. The study was registered with the National Institutes of Health (NIH) of the Ministry of Health (MOH), Malaysia (available at www.nmrr.gov.my, National Medical Research Register

(NMRR) number: NMRR-13-524-16614). The study was evaluated and approved by the Human Research Ethics Committee (HREC), Universiti Sains Malaysia (USM) in Kelantan, Malaysia (Reference number: USMKK/PPP/JEPeM (253.3 [14]).

Assessment of subjective sleep quality using the Malay version of the Pittsburgh Sleep Quality Index (PSQI-M)

Subjective sleep quality was measured by the Malay version of the Pittsburgh Sleep Quality Index (PSQI–M) (Buysse *et al.*, 1989). The PSQI is a validated questionnaire to measure subjective sleep quality and disturbances during the previous month that has been translated into several languages including Malay. The Malay translation of this questionnaire was performed by MAPI Research Trust and permission for use the Malay version of the Pittsburgh Sleep Quality Index – PSQI was obtained from the author at the University of Pittsburgh (Buysse *et al.*, 1989). The PSQI contains 19 items that are included in scoring. The 19 individual items are used to generate seven component scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medications, and daytime dysfunction. Each of the seven component scores is determined based on scoring guidelines, with the seven component scores each with a potential range of 0–3, where '3' reflects the negative extreme on the Likert Scale. The sum of these seven component scores yields one global score of subjective sleep quality with a potential range of 0–21, with higher scores represent poorer subjective sleep quality (i.e. a global PSQI score of > 5 is associated with poor sleep quality) (Buysse *et al.*, 1989).

Genotyping methods for detection of *OPRM1* polymorphisms

Subjects provided 5 mL venous blood sample, and genomic DNA was extracted from 200 µl of whole blood by use of the QIAamp® DNA Blood Mini Kit (Qiagen Gmbh, Hilden, Germany). Allele-specific multiplex polymerase chain reaction (PCR) was used to detect *OPRM1* polymorphisms [118A>G (dbSNP rs1799971) and IVS2+691G>C (dbSNP rs2075572)] (Mohamed Nazar, 2013; Zahari *et al*., 2015; Zahari *et al.*, 2016b, Zahari *et al.*, 2016a). All reactions were performed on the Applied Biosystems® Veriti® 96-Well Thermal Cycler (Applied Biosystems, USA). A complete PCR method is available upon request.

Statistical analysis

Sample size was calculated prior the start of the study based on the Cohen sample size table (Cohen, 1992), using medium population effect size (ES) of 0.50 assuming a two-tailed 5% type I error rate and 80% power. The required sample size per group was 64 alleles or subjects for comparisons of means of two groups (under allelic additive model, genotype dominant and recessive model). *OPRM1* haplotypes and diplotypes were constructed based on the expectation-maximization (EM) algorithm using the population genetic data analytical program, Golden Helix SNP and Variation Suite 7 (SVS 7, version 7.3.1; Golden Helix Inc., Bozeman, MT, USA). The sum of seven component scores of the PSQI was calculated as the global score for subjective sleep quality. Means and standard deviations were calculated for the global score of the PSQI. Independent t-test and one-way ANOVA test were used to compare the mean global PSQI scores between *OPRM1* polymorphisms (118A>G and IVS2+691G>C) according to their genotypes and allelic additive models, genotype dominant and recessive models, haplotypes and diplotypes where appropriate. Haplotypes and diplotypes with frequencies less than 10% were pooled, since rare or minor haplotypes and diplotypes provide little additional information while increasing the degrees of freedom. Data analyses were done after all genotyping of the subjects was completed. There were no missing data for all the subjects included in this study. Given the exploratory nature of this study and only one gene was tested (Belfer *et al.*, 2013), correction for multiple testing was not performed for the exploratory statistical analyses. Statistical analysis was carried out using SPSS/Win software (Version 22, SPSS, Inc., Chicago, IL, USA). A p value < 0.05 was considered significant.

RESULTS

Descriptive statistics

A total of 161 opioid-naive individuals fulfilled inclusion and exclusion criteria, gave informed consent and completed the study. The mean age of study participants was 27.74 years [standard deviation (SD) = 10.32; range: 18−63 years]. The mean body mass index (BMI) was 24.82 kg/m^2 (SD = 5.33; range: 15−45 kg/m^2). The mean PSQI score was 4.76 (SD = 2.31, range 0–13), slightly below a cut-off score of 5, thus indicating good overall sleep quality (Buysse *et al.*, 1989). Specifically, 68.9% (N = 111) had PSQI scores of ≤ 5, indicating they were 'good sleepers'.

OPRM1 polymorphisms

The *118A/G* and *IVS2+691G/C* alleles of *OPRM1* were successfully amplified from the DNA of 161 opioid-naive subjects. The allele frequencies for *118G* and *IVS2+691C* were 53.1% and 85.1%, respectively. The combination of the individual polymorphisms into *OPRM1* haplotype pairs revealed the presence of 7 diplotypes. The most common haplotype pair was AC/GC (*N* = 54, 33.5%), followed by GC/GC (*N* = 45, 28.0%), GC/AG (*N* = 23, 14.3%), and AC/AG (*N* = 21, 13.0%).

Association of *OPRM1* polymorphisms with PSQI scores

Table I shows that subjects with homozygous 118 AA genotype had lower PSQI scores compared to those without 118 AA genotype (118 AG/GG genotype) (4.30 *vs* 4.90), but the difference was not statistically significant (p = 0.167).

Subjects with the heterozygous IVS2+691 GC genotype had the highest PSQI scores among the three IVS2+691G>C genotypes (Table I). Subjects with the *IVS2+691G* allele (IVS2+691 GG/GC genotype) had higher PSQI scores compared to those without the allele (IVS2+691 CC genotype) (5.26 *vs* 4.55), but the difference again did not reach statistical significance (p = 0.079).

In view of this, we performed haplotype and diplotype analysis constructed from the two *OPRM1* polymorphisms. Haplotype analysis revealed a significant difference of the mean PSQI scores between subjects with AC haplotype and those without this haplotype

TABLE I - Association between 118A>G and IVS2+691G>C polymorphisms and PSQI scores in opioid-naive Malay Males

Polymorphism	*N*	(%)	Mean	SD	Test statistic (df)	*p* value[c]
118A>G						
Genotype (*N* = 161)						
AA	37	23.0	4.30	1.61	1.11 (2, 158)[a]	0.331
AG	77	47.8	4.81	2.22		
GG	47	29.2	5.04	2.84		
Allele (*N* = 322)						
A	151	46.9	4.56	1.95	-1.50 (313)[b]	0.134
G	171	53.1	4.94	2.57		
Dominant model						
AA	37	23.0	4.30	1.61	-1.39 (159)[b]	0.167
AG + GG	124	77.0	4.90	2.47		
Recessive model						
AA + AG	114	70.8	4.64	2.05	-0.88 (67)[b]	0.382
GG	47	29.2	5.04	2.84		
IVS2+691G>C						
Genotype (*N* = 161)						
GG	1	0.6	4.00	-	1.71 (2, 158)[a]	0.184
GC	46	28.6	5.28	2.34		
CC	114	70.8	4.55	2.28		
Allele (*N* = 322)						
G	48	14.9	5.23	2.31	1.54 (320)[b]	0.124
C	274	85.1	4.68	2.30		
Dominant model						
GG	1	0.6	4.00	-	-0.33 (159)[b]	0.743
GC + CC	160	99.4	4.76	2.31		
Recessive model						
GG + GC	47	29.2	5.26	2.33	1.77 (159)[b]	0.079
CC	114	70.8	4.55	2.28		

TABLE I - Association between 118A>G and IVS2+691G>C polymorphisms and PSQI scores in opioid-naive Malay Males (cont.)

Polymorphism	*N*	(%)	Mean	SD	Test statistic (df)	*p* value[c]
Haplotype (*N* = 322)[d]						
GC	169	52.5	4.92	2.55	2.66 (3, 318)[a]	0.048
AC	105	32.6	4.29	1.76		
AG	46	14.3	5.17	2.22		
GG	2	0.6	6.50	4.95		
GC	169	52.5	4.92	2.55	3.68 (2, 319)[a]	0.026
AC	105	32.6	4.29	1.76		
Combined AG and GG	48	14.9	5.23	2.31		
GC	169	52.5	4.92	2.55	1.32 (313)[b]	0.187
Not GC	153	47.5	4.58	1.99		
AC	105	32.6	4.29	1.76	-2.90 (278)[b]	0.004
Not AC	217	67.4	4.99	2.50		
AG	46	14.3	5.17	2.22	1.33 (320)[b]	0.186
Not AG	276	85.7	4.69	2.31		
Diplotype (*N* = 161)						
AC/GC	54	33.5	4.28	1.89	2.83 (4, 156)[a]	0.027
GC/GC	45	28.0	4.98	2.79		
GC/AG	23	14.3	6.04	2.48		
AC/AG	21	13.0	4.33	1.62		
Others[e]	18	11.2	4.50	2.09		
AC/GC	54	33.5	4.28	1.89	-1.89 (159)[b]	0.061
Not AC/GC	107	66.5	5.00	2.46		
GC/GC	45	28.0	4.98	2.79	0.66 (64)[b]	0.509
Not GC/GC	116	72.0	4.67	2.10		
GC/AG	23	14.3	6.04	2.48	2.96 (159)[b]	0.004
Not GC/AG	138	85.7	4.54	2.22		
AC/AG	21	13.0	4.33	1.62	-0.90 (159)[b]	0.368
Not AC/AG	140	87.0	4.82	2.39		

N, number of subject/allele/haplotype/diplotype; SD, standard deviation. [a] *F*-statistic using one-way ANOVA test; [b] *t*-statistic using independent t-test; [c] *p* value is significant at < 0.05; [d] Haplotype patterns were constructed from the two *OPRM1* polymorphisms (118A>G and IVS2+691G>C); [e] Diplotype with frequency less than 10.0% was pooled under 'others' (included AG/GG and GC/GG)

[*t* (278) = -2.90, *p* = 0.004]. Subjects with the AC haplotype had significantly lower PSQI scores compared to those without the haplotype (4.29 *vs* 4.99).

There was also a significant difference of the mean PSQI scores between subjects with the combined heterozygous genotype (GC/AG diplotype) and those without this diplotype (p = 0.004). Subjects with the GC/AG diplotype had significantly higher PSQI scores compared to those without the diplotype (6.04 *vs* 4.54).

DISCUSSION

The present study aimed to investigate the associations between *OPRM1* polymorphisms and sleep quality in opioid-naive population. This study revealed that the mean PSQI scores was significantly lower in carriers of the AC haplotype than those without the haplotype. On the other hand, this study found that the mean PSQI scores was significantly higher in carriers of the GC/AG diplotype than those without the diplotype. From these data, it is suggested that variations in *OPRM1* may influence sleep quality even in the opioid-naive population. Previous research using the classical twin design has highlighted the contribution of genes to subjective sleep quality (Genderson *et al.*, 2013). Sleep quality was measured using the PSQI in 1218 middle-aged twin men from the

Vietnam Era Twin Study of Aging (VETSA) (mean age = 55.4 years; range: 51–60). They found that 34% of variability in the global PSQI score was due to additive genetic effects (heritability). Similarly, the heritability of poor sleep (i.e. a dichotomous measure based on the cut-off of global PSQI > 5) was 31%. Previous molecular genetic studies have begun to identify genetic variations related to sleep–wake behavior includes 3111 T/C SNP (rs1801260) of the Circadian Locomotor Output Cycles Kaput (CLOCK) gene (Benedetti *et al.*, 2007) and variable-number tandem-repeat (VNTR) polymorphism of the period circadian clock 3 gene (PER3) (Viola *et al.*, 2007). Also, there are a number of recent genome-wide association analyses (GWAS) studies on sleep quality and quantity (Lane *et al.*, 2017; Jones *et al.*, 2016; Byrne *et al.*, 2013).

Among the identified polymorphisms within the OPRM1 gene, 118A>G polymorphism is the most frequently studied in the literatures. An *in vitro* study has demonstrated that the *118G* allele altered the β-endorphin binding affinity (Bond *et al.*, 1998). The *118G* allele showed a 3-fold increase in β-endorphin binding at the receptor than the most common allelic form of the receptor (Bond *et al.*, 1998). β-endorphin involves in the regulation of sleep and wakefulness, and administration of β-endorphin has been shown to produce insomnia (King *et al.*, 1981). It is possible, therefore, the *118G* allele carriers have higher β-endorphin binding at the OPRM1 and consequently, resulted in poorer sleep quality compared to those without the allele. In our study, although results did not reach statistical significance, *118G* allele carriers (118 AG/GG genotype) had higher PSQI scores when compared with the *118G* allele non-carriers (118 AA genotype).

A more significant influence of *OPRM1* polymorphisms on sleep quality was observed under haplotype and diplotype analysis. We found that subjects with AC haplotype (i.e. carriers of combined *118A* and *IVS2+691G* alleles) had 14% significantly lower PSQI scores compared to non-carriers of this haplotype. We also found that subjects with GC/AG diplotype (i.e. carriers of combined 118 AG and IVS2+691 GC genotypes) had 33% significantly higher PSQI scores compared to non-carriers of this diplotype. Unfortunately, the exact molecular mechanism regarding the effects of AC haplotype and GC/AG diplotype on the sleep quality cannot be determined from the current study. To the best of our knowledge, data on the influence of the *OPRM1* haplotype and diplotype on sleep quality among opioid-naive individuals is not available for reference. However, previous studies have indicated that the function of the OPRM1 is under the influence OPRM1 polymorphisms (Xin, Wang, 2002; Hoehe *et al.*, 2000; Lötsch, Geisslinger, 2006). Based on our results, we suggest that the AC haplotype and GC/AG diplotype may affect OPRM1 expression or function (or both), and resulted in altered binding affinity between endogenous opioid peptides and the OPRM1, and hence haplotype and diplotype differences may contribute to inter-individual differences in sleep-wakefulness effects of endogenous opioid peptide β-endorphin.

It has been previously demonstrated that the AC/AG diplotype for the 118A>G and IVS2+691G>C polymorphisms of *OPRM1* is associated with better sleep quality among opioid-dependent patients on MMT (Zahari *et al.*, 2016a). However, in opioid-naive individuals, no significant difference of the mean PSQI scores was observed between subjects with the AC/AG diplotype and those without this diplotype ($p = 0.368$). It is highly possible that the OPRM1 affects sleep by acting on different mechanisms in opioid-naive individuals and opioid-dependent patients (Wang *et al.*, 2012; García-García, Drucker-Colín, 1999). At this juncture, it is difficult to explain the differential effects on sleep quality among opioid-naive individuals and opioid-dependent patients with these polymorphisms and further studies are needed.

The findings of the present study have therapeutic implications. The main findings here were that the contribution of *OPRM1* polymorphisms influences to the sleep quality among opioid-naive individuals varied between genotype, haplotype and diplotype analysis. Most notably that subjects with GC/AG diplotype ($N = 23$, 14.3%) had the PSQI scores of > 5, indicating they were 'poor sleepers', and their PSQI scores was significantly higher than those without the diplotype. These results provide an initial prediction on sleep quality for individuals with GC/AG diplotype which enables prescription of sleep medications for individuals with the diplotype. Furthermore, the clinician may use genotyping results of *OPRM1* in making decision about pharmacotherapeutic strategies in terms of the choice of medications to be prescribed, and dose and/or route of administration of drugs for treatment of postoperative sleep disturbance among patients undergoing surgery. Indeed, this study demonstrated that individuals with GC/AG diplotype are more susceptible to poor sleep compared to individuals without the diplotype. It is possible that the good sleep is more difficult to be achieved in individuals with GC/AG diplotype. Thus, the results of the current study provide new information regarding the genetic factors that may be consider when establishing clinical recommendations or a suitable treatment protocol for providing sleep disorder treatment and management. However, separate analysis of individual components of the

PSQI showed that only sleep disturbance was significantly associated with GC/AG diplotype [t (df) = 2.14 (26.8), p = 0.042]. Individuals with GC/AG diplotype had 25% higher sleep disturbance score compared to individuals without the diplotype (1.35 *vs* 1.08). Early detection and intervention of sleep disturbances can help reduce the morbidity and mortality associated with this and helps increase patient's quality of life.

Certain limitations of this study must be recognized. This study did not use an objective sleep evaluation test such as polysomnography (PSG) and did not obtain data on serum concentrations of β-endorphin and supporting data on the functional effects of *OPRM1* polymorphisms (118A>G and IVS2+691G>C), haplotypes or diplotypes on OPRM1 expression or function (or both). Only males were included but we aimed to reduce the confounding effects of gender on sleep quality (Fatima *et al.*, 2016). In view that correction for multiple comparisons was not performed for exploratory statistical analyses, caution is required when reviewing these analyses. The sample size was another limitation, but we considered our study as more explorative in nature and replication by other investigators and further studies with larger sample sizes are encouraged. This study lacked a multivariate approach. Non-genetics sleep-related factors were not reported, and this study was design to exclude subjects with chronic medical and psychiatric illness such as chronic pain, depression and anxiety that are associated with sleep disorder because our focus was to look into pharmacogenetics factors associated with sleep quality among opioid-naive individuals. However, we did not find any significant differences between individuals with GC/AG diplotype and those without the diplotype in terms of age and BMI, therefore they were well matched with respect to age and BMI.

Further studies are needed to study other *OPRM1* polymorphisms and genetic variations of other sleep related-genes, and to obtain data on endogenous β-endorphin concentration and data on the functional effects of AC haplotype and GC/AG diplotype on OPRM1 expression and/or function.

In summary, our study indicates that the AC haplotype and GC/AG diplotype for the 118A>G and IVS2+691G>C polymorphisms of *OPRM1* are associated with better and poorer sleep quality, respectively among opioid-naive individuals. Our study may be considered explorative in nature and it would require replication by other investigators. Nevertheless, these results provide a starting point for a better understanding of genetic contributions to sleep quality which may help to improve diagnosis and treatment of sleep disturbance.

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