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ORIGINAL RESEARCH

The AC/AG Diplotype for the 118A>G and IVS2 + 691G>C Polymorphisms of OPRM1 Gene is Associated with Sleep Quality Among Opioid-Dependent Patients on Methadone Maintenance Therapy

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ABSTRACT

Introduction: Methadone is a full agonist of the opioid receptor mu 1 which is encoded by the OPRM1 gene. Sleep disorders were frequently reported by opioid-dependent patients during methadone maintenance therapy (MMT). It is possible, therefore, that genetic polymorphisms in *OPRM1* influence sleep quality among patients on MMT. This study investigated the association of *OPRM1* polymorphisms with sleep quality among opioid-dependent patients on MMT.

Methods: The sleep quality of 165 male opioid-dependent patients receiving MMT was evaluated using the Pittsburgh Sleep Quality Index (PSQI). DNA was extracted from whole blood and subjected to polymerase chain reaction (PCR) genotyping.

Results: Patients with IVS2 + 691 CC genotype had higher PSQI scores [mean (SD) = 5.73 (2.89)] compared to those without the IVS2 + 691 CC genotype (IVS2 + 691 GG/GC

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genotype) [4.92 (2.31)], but the difference did not reach statistical significance ($p = 0.081$). Patients with combined 118 AA genotype and IVS2 + 691 GC genotype (AC/AG diplotype) had significantly lower PSQI scores [mean (SD) = 4.25 (2.27)] compared to those without the diplotype [5.68 (2.77)] ($p = 0.018$).

Conclusion: Our study indicates that the AC/AG diplotype for the 118A>G and IVS2 + 691G>C polymorphisms of OPRM1 gene is associated with better sleep quality among males with opioid dependence on MMT.

Keywords: AC/AG diplotype; Male patients; Methadone; Methadone maintenance therapy; Opioid dependence; Opioid receptor; Opioid receptor, mu 1 gene; OPRM1; Pittsburgh Sleep Quality Index; Sleep quality

INTRODUCTION

Sleep disorders were frequently reported by opioid-dependent patients during methadone maintenance therapy (MMT) [1–6]. Patients on MMT demonstrated disrupted sleep including increased stage 2 sleep and decreased REM sleep and stage 1 sleep compared to age, sex, and body mass index (BMI) matched normal subjects [7]. In addition, they reported significantly worse daytime function, were more depressed, and had increased daytime sleepiness when compared to the control subjects [8]. The sleep disturbances among opioid-dependent patients on MMT contributed to premature exit from treatment [9–11], increased use of medications (prescribed or "over the counter") to help with sleep [1–3], and increase in chronic depressive symptoms [8, 12]. The sleep disturbances also impacted on quality of life and could impair engagement with treatment leading to continued drug use [1, 5, 12].

Previous studies showed that opioids affected sleep by acting on both sleep- and wake-promoting systems at the pontine reticular formation (PRF) and the substantia innominata within the basal forebrain (BF) [13]. In the PRF, the administration of opioids decreased adenosine levels and this was dependent upon opioid receptor, mu 1 gene (OPRM1) agonism, and subsequently resulted in sleep disturbance-related side effects of opioids [14].

Opioid drugs, including morphine, fentanyl, and methadone, are agonists of the μ-opioid receptor which is encoded by the OPRM1 gene. It has been suggested that the efficacy and side effects of commonly used opioids are associated with their affinity for μ-opioid receptor. Some of the *OPRM1* polymorphisms that affect the density and function and consequently the signaling efficacy of μ-opioid receptors may contribute to interindividual variations in the response to opioids [15–17]. 118A>G (dbSNP rs1799971, Asn40Asp) polymorphism is one of the most frequently studied polymorphisms of *OPRM1*. It is found in exon 1 and may greatly affect the μ-opioid receptor *N*-glycosylation and reduced stability of the receptor in cell cultures [18]. *N*-Glycosylation plays a part in many cellular processes like receptor folding, sorting, expression, and ligand binding. IVS2 + 691G>C (dbSNP rs2075572) polymorphism at 691 bp downstream of exon 2 is located within intron 2 [19–21]. The polymorphism might change the regulation of the expression of OPRM1 gene and might also cause formation of different isoforms of human μ-opioid receptor [20, 22].

A few studies have reported on the association between methadone treatment and *OPRM1* polymorphisms [23–27]. Thus far, one study explored if the *OPRM1* polymorphisms could provide a possible explanation for the

noted sleep problems of opioid-addicted individuals [24]; unfortunately the role of the *OPRM1* individual's pair of haplotypes (diplotype) remains uncertain because they did not consider analysis of *OPRM1* diplotype.

Recently, we published our data on the more clinically important aspects of pain sensitivity [28]. We felt that it is also necessary to report our findings on the relationship between sleep quality and opioid genetic polymorphisms. Thus, in the present paper, our aim was to investigate sleep quality as sleep disorders were frequently reported by opioid-dependent patients during MMT. A better understanding of the role of *OPRM1* polymorphisms in sleep disturbance-related side effects of opioids has implications for the treatment of sleep and addictive disease, and clinical management of each in the presence of the other. To help resolve this we aimed to investigate the influence of *OPRM1* polymorphisms on sleep quality among opioid-dependent patients on MMT.

METHODS

Patients

Opioid-dependent patients who had been diagnosed according to *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) criteria [29] and were attending the national MMT programme at the Hospital Universiti Sains Malaysia and other MMT clinics in Kota Bharu, Pasir Mas, Pasir Puteh and Bachok, Kelantan, Malaysia during the study period were screened and invited to participate in the study. In this study, we included only men as this reflects the cohort population of drug abusers in Malaysia where more than 90% of them are male [30].

Inclusion criteria were (a) men aged more than 18 years; (b) those stabilized in treatment, defined as having been enrolled in the MMT programme for more than 1 month; (c) patients with two consecutive negative urine tests 1 week prior to the study; (d) free from acute medical, surgical, and psychiatric illnesses; (e) free from regular use of alcohol; and (f) free from drug intoxication. The exclusion criteria were (a) individuals with major psychiatric illnesses such as schizophrenia; (b) individuals who were currently illicitly taking benzodiazepines, cannabinoids, and barbiturates; (c) individuals on regular anticonvulsants, neuroleptics, or analgesics; (d) individuals with chronic or ongoing acute pain; (e) individuals with a history of analgesics ingestion within 3 days before the study; and (f) individuals with severe cognitive impairment which may interfere with sleep assessments and/or communication.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2013. Written informed consent was obtained from each subject after a complete description of the study. The study was approved by the Human Research Ethics Committee (HREC), Universiti Sains Malaysia (USM) in Kelantan, Malaysia (reference number USMKK/PPP/JEPeM (253.3 [14])) and the Medical Research and Ethics Committee (MREC) at the Ministry of Health (MOH), Malaysia (reference number NMRR-13-524-16614).

Assessment of Sleep Quality Using Malay Version of the Pittsburgh Sleep Quality Index (PSQI-M)

Patients were asked to fill out the Malay version of the Pittsburgh Sleep Quality Index (PSQI–M). The PSQI is a validated questionnaire to measure subjective sleep quality and disturbances during the previous month and it has been translated into several languages including Malay. The PSQI has been successfully used in patients with opioid dependence receiving MMT among ethnically different populations [1–4, 6, 8, 31–38]. The 19 individual items are used to generate seven component scores: subjective sleep quality (one item), sleep latency (two items), sleep duration (one item), habitual sleep efficiency (three items), sleep disturbances (nine items), use of sleep medications (one item), and daytime dysfunction (two items). Each of the seven component scores is determined on the basis of 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 representing poorer subjective sleep quality [39].

Genotyping Methods for *OPRM1* Polymorphisms

Genomic DNA was extracted from whole blood by use of the QIAamp® DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). *OPRM1* polymorphisms [118A>G (dbSNP rs1799971) and IVS2 + 691G>C (dbSNP rs2075572)] were determined by use of an allele-specific multiplex polymerase chain reaction (PCR) method [28, 40]. 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

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) was used to apply the expectation–maximization (EM) algorithm to construct *OPRM1* haplotypes and diplotypes. The sum of seven component scores of PSQI was calculated as the global score for sleep quality. Independent *t* test and one-way ANOVA test were used to compare PSQI scores (i.e., continuous variable) [1, 6] 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 (frequencies less than 10.0% were pooled). Data analyses were done after all genotyping of the patients was completed. Statistical analysis was carried out using SPSS/Win software (Version 22, SPSS, Inc., Chicago, IL, USA). Correction for multiple testing was not performed since only one gene was tested [41]. A p value less than 0.05 was considered significant.

RESULTS

Characteristics of Study Participants

A total of 165 opioid-dependent patients fulfilled inclusion and exclusion criteria, gave informed consent, and completed the study. Patients' mean age was 37.27 years [standard deviation (SD) 6.24, range 25–55]. The mean duration in the MMT program was 2.92 years

(SD 2.09, range 0.33–9.00). The mean daily methadone dose was 76.64 mg/day (SD 37.63, range 20–360). The mean PSQI score was 5.47 (SD 2.74, range 0–14), slightly above a cutoff score of 5, thus indicating poor overall sleep quality [39]. Specifically, 58.8% ($N = 97$) of patients had PSQI scores greater than 5, indicating they were 'poor sleepers'.

OPRM1 Polymorphisms

The observed allelic frequencies were 40.6% for 118G and 83.9% for IVS2 + 691C. The combination of the individual polymorphisms into *OPRM1* haplotype pairs revealed the presence of seven diplotypes. The most common haplotype pair was AC/GC ($N = 56$, 33.9%), followed by AC/AC ($N = 34$, 20.6%) and AC/AG ($N = 24$, 14.5%).

Association of *OPRM1* Polymorphisms with PSQI Scores

Table 1 shows that patients with homozygous 118 AA genotype had lower PSQI scores compared to those without 118 AA genotype (118 AG/GG genotype) (5.28 vs 5.58), but the difference did not quite reach statistical significance ($p = 0.499$).

Patients with the heterozygous GC genotype had the lowest PSQI scores among the three IVS2 + 691G>C genotypes (Table 1). Patients with the homozygous CC genotype had higher PSQI scores when compared to those without the IVS2 + 691 CC genotype (IVS2 + 691 GG/GC genotype) (5.73 vs 4.92), but the difference did not reach statistical significance ($p = 0.081$).

In view of this, we performed diplotype analysis constructed from the two *OPRM1* polymorphisms (118A>G and IVS2 + 691G>C) and found a significant difference between patients with combined homozygous 118 AA genotype and heterozygous IVS2 + 691 GC genotype (AC/AG diplotype) and those without this diplotype ($p = 0.018$). Patients with the AC/AG diplotype had significantly lower PSQI scores when compared with those without the diplotype (4.25 vs 5.68).

DISCUSSION

Previous association studies have produced mixed results regarding *OPRM1* polymorphisms and methadone treatment efficacy, and side effects included changes in libido and insomnia [24], in MMT response status in terms of illicit opioid use detection in random urinalysis [25], in apparent susceptibility to methadone poisoning [26, 27], and in both pain responses and opioid addiction [23]. Among studies that focused on genetic polymorphisms related to pharmacodynamics of methadone, only one study investigated the association between the noted sleep problems of opioid-addicted individuals and polymorphisms in gene coding for the OPRM1 [24]; unfortunately the role of the *OPRM1* individual's pair of haplotypes (diplotype) remains uncertain because they did not consider analysis of *OPRM1* diplotypes.

Previously, Wang et al. [24] found that the insomnia side effect was significantly higher in patients with IVS2 + 691 CC genotype than those without the genotype (IVS2 + 691 GG/GC genotype). Their results suggested that IVS2 + 691G>C polymorphism may participate in the regulation of the function of the OPRM1. Interestingly, in the current study, PSQI scores were 16.3% higher in patients with homozygous IVS2 + 691 CC genotype than those without the genotype (IVS2 + 691 GG/GC genotype); however, our results were statistically insignificant ($p = 0.081$). Additionally, when the

Table 1 Association between 118A>G and IVS2 + 691G>C polymorphisms and PSQI scores in opioid-dependent patients

Polymorphism	Number	Percent	Mean	SD	Test statistic (*df*)	*p* value[c]
118A>G						
Genotype ($N = 165$)						
AA	58	35.2	5.28	2.83	0.36 (2, 162)[a]	0.698
AG	80	48.5	5.50	2.71		
GG	27	16.4	5.81	2.70		
Allele ($N = 330$)						
A	196	59.4	5.37	2.77	−0.85 (328)[b]	0.399
G	134	40.6	5.63	2.69		
Dominant model						
AA	58	35.2	5.28	2.83	−0.68 (163)[b]	0.499
AG + GG	107	64.8	5.58	2.70		
Recessive model						
AA + AG	138	83.6	5.41	2.75	−0.71 (163)[b]	0.480
GG	27	16.4	5.81	2.70		
IVS2 + 691G>C						
Genotype ($N = 165$)						
GG	1	0.6	9.00	–	2.71 (2, 162)[a]	0.070
GC	51	30.9	4.84	2.26		
CC	113	68.5	5.73	2.89		
Allele ($N = 330$)						
G	53	16.1	5.00	2.35	−1.37 (328)[b]	0.170
C	277	83.9	5.56	2.80		
Dominant model						
GG	1	0.6	9.00	–	1.29 (163)[b]	0.198
GC + CC	164	99.4	5.45	2.74		
Recessive model						
GG + GC	52	31.5	4.92	2.31	−1.76 (163)[b]	0.081
CC	113	68.5	5.73	2.89		
Haplotype ($N = 330$)[d]						
AC	148	44.8	5.51	2.89	0.76 (3, 326)[a]	0.518
GC	129	39.1	5.63	2.70		
AG	48	14.5	4.94	2.34		

Table 1 continued

Polymorphism	Number	Percent	Mean	SD	Test statistic (*df*)	*p* value[c]
GG	5	1.5	5.60	2.70		
AC	148	44.8	5.51	2.89	1.01 (2, 327)[a]	0.366
GC	129	39.1	5.63	2.70		
Combined AG and GG	53	16.1	5.00	2.35		
AC	148	44.8	5.51	2.89	0.20 (328)[b]	0.839
Not AC	182	55.2	5.45	2.62		
GC	129	39.1	5.63	2.70	0.82 (328)[b]	0.410
Not GC	201	60.9	5.37	2.76		
AG	48	14.5	4.94	2.34	−1.47 (328)[b]	0.143
Not AG	282	85.5	5.56	2.79		
Diplotype ($N = 165$)						
AC/GC	56	33.9	5.45	2.91	1.40 (5, 159)[a]	0.229
AC/AC	34	20.6	6.00	2.98		
AC/AG	24	14.5	4.25	2.27		
GC/AG	23	13.9	5.48	2.17		
GC/GC	23	13.9	6.00	2.78		
Others[e]	5	3.0	5.60	2.70		
AC/GC	56	33.9	5.45	2.91	−0.09 (163)[b]	0.930
Not AC/GC	109	66.1	5.49	2.67		
AC/AC	34	20.6	6.00	2.98	1.26 (163)[b]	0.209
Not AC/AC	131	79.4	5.34	2.67		
AC/AG	24	14.5	4.25	2.27	−2.40 (163)[b]	0.018
Not AC/AG	141	85.5	5.68	2.77		
GC/AG	23	13.9	5.48	2.17	0.01 (163)[b]	0.992
Not GC/AG	142	86.1	5.47	2.83		
GC/GC	23	13.9	6.00	2.78	0.99 (163)[b]	0.322
Not GC/GC	142	86.1	5.39	2.74		

N number of subject/allele/haplotype/diplotype, *SD* standard deviation

[a] *t* statistic using independent *t* test

[b] *F* statistic using one-way ANOVA 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' (and included AG/GG and GC/GG)

PSQI scores were compared using the diplotype approach, we found that patients with combined 118 AA genotype and IVS2 + 691 GC genotype (AC/AG diplotype) had 25.2% significantly lower PSQI scores compared to those without the diplotype. To the best of our knowledge, data on the influence of the *OPRM1* diplotype on sleep quality among opioid-dependent patients is not available for reference. However, this finding suggested that opioid-related adverse effects such as sleep problems were less likely to occur in patients with AC/AG diplotype at 118 and IVS2 + 691 in *OPRM1*, given that a mechanism of opioid-induced adverse events involves OPRM1. Hence, results of the current study support the suggestion by Wang et al. [24] that the OPRM1 gene and its transcript isoforms may be involved in the underlying cause of insomnia.

The exact molecular mechanism regarding the effects of AC/AG diplotype on the sleep quality is unclear. However, it is well established that, in general, the function of the OPRM1 is under the influence OPRM1 gene polymorphisms [19–21]. The IVS2 + 691G>C polymorphism is predicted to change the affinity of transcriptional regulatory factors for the intronic DNA sequence and directly alter mRNA levels, and therefore it might change the regulation of OPRM1 gene expression. It was also shown that the DNA intronic sequence can be involved in alternative DNA splicing, resulting in different isoforms of human OPRM1 [20, 22].

On the basis of our results, we suggest that strong linkage disequilibrium (LD) between these polymorphisms and other unstudied polymorphisms [42, 43] formed a series of diplotypes which may affect OPRM1 expression or function (or both) at the site of its action in the brain and resulted in altered binding affinity between endogenous (and/or exogenous) opioid agents and the OPRM1, and hence diplotype differences may contribute to interindividual differences in sleep-disrupting effects of opioids.

Available data indicates that opioids affect sleep by acting on both sleep- and wake-promoting systems at the pontine reticular formation (PRF) and the substantia innominata within the basal forebrain (BF) [13]. Opioids decreased adenosine levels in the PRF and this is dependent upon OPRM1 agonism, and subsequently resulted in sleep disturbance-related side effects of opioids [14]. Our results support the hypothesis that patients with AC/AG diplotype had a higher ability to prevent opioid-induced decreases in adenosine and therefore resulted in lower susceptibility to sleep disturbances.

Some limitations to this study need to be highlighted. In our current study, we excluded patients with psychiatric illnesses such as schizophrenia, depression, and anxiety that are commonly associated with sleep disorder; the presence of these illnesses would be expected to increase the severity of sleep problems in our study subjects. Interestingly, although known psychiatric illnesses were one of our exclusion criteria, no participants were excluded because of this criterion. Non-genetic sleep-related factors among patients on MMT were not reported because the focus of the current manuscript was to look into pharmacogenetics factors associated with susceptibility to opioid-induced sleep disturbance among opioid-dependent patients on opioid maintenance therapy.

Patients without the AC/AG diplotype ($N = 141$, 85.5%) had a mean PSQI score of 5.68 (SD 2.77), slightly above a cutoff score of 5, thus indicating poor overall sleep quality among them [39]. We suggest that sleep disorders should be evaluated and treated among MMT patients, particularly in those without the AC/AG diplotype. Patients with

sleep disorders tend to self-medicate to promote sleep or to stay awake during the day [44]. Many of these patients may be at risk of significant drug–drug interactions resulting in ineffective treatment and enhanced side effects of the drugs which could have a profound impact on quality of life, health, and even could impair engagement with treatment leading to continued drug use [1, 5, 12].

Further studies are needed to study other *OPRM1* polymorphisms and genetic variations of other sleep-related genes and genes related to pharmacokinetics and pharmacodynamics of methadone, and to obtain data on endogenous adenosine concentration and data on the functional effects of AC/AG diplotype on OPRM1 expression or function in the brain.

CONCLUSION

Our study indicates that the AC/AG diplotype for the 118A>G and IVS2 + 691G>C polymorphisms of OPRM1 gene is associated with better sleep quality among opioid-dependent patients on MMT. The results of this study lead to a better understanding of the pharmacogenetics factors associated with susceptibility to opioid-induced sleep disturbance in this population. Therefore, determining which diplotyes may increase susceptibility to sleep disturbance-related side effects of methadone could be important. Personalised medicine based on pharmacogenetics may be able to improve the effectiveness of methadone and reduce its side effects.

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Compliance with Ethics Guidelines. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2013. The study procedures were approved by the Human Research Ethics Committee (HREC), Universiti Sains Malaysia (USM) in Kelantan,

Malaysia (reference number USMKK/PPP/JEPeM (253.3 [14])) and the Medical Research and Ethics Committee (MREC) at the Ministry of Health (MOH), Malaysia (reference number NMRR-13-524-16614). Informed consent was obtained from all individual participants included in the study.

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