Influence of DRD2 Polymorphisms on the Clinical Outcomes of Opioid-dependent Patients on Methadone Maintenance Therapy

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Introduction: Dopamine receptor D2 (DRD2) is one of the dopamine receptors that have been studied in relation to opioid dependence. It is possible, therefore, that DRD2 gene (DRD2) polymorphisms influence treatment outcomes of patients with opioid dependence. The objective of this study was to investigate the influence of DRD2 polymorphisms on the clinical outcomes of opioid-dependent patients on methadone maintenance therapy (MMT). Materials and Methods: Patients with opioid dependence (n = 148) were recruited from MMT clinics. Pain sensitivity, severity of the opiate withdrawal syndrome, and sleep quality were assessed using cold pressor test (CPT), Subjective Opiate Withdrawal Scale (SOWS-M), and Pittsburgh Sleep Quality Index (PSQI)-Malay, respectively. Deoxyribonucleic acid (DNA) was extracted from whole blood, and then was used for genotyping of Val96Ala, Leu141Leu, Val154Ile, Pro310Ser, Ser311Cys, TaqIA, -141C Ins/Del, and A-241G polymorphisms. Results: Among 148 patients, 8.1% (n = 12), 60.8% (n = 90), 27.7% (n = 41), and 29.1% (n = 43) had at least one risk allele for Ser311Cys, TaqIA, -141C Ins/Del, and A-241G polymorphisms. Results: Among 148 patients, 8.1% (n = 12), 60.8% (n = 90), 27.7% (n = 41), and 29.1% (n = 43) had at least one risk allele for Ser311Cys, TaqIA, -141C Ins/Del, and A-241G polymorphisms, respectively. There were no significant differences in pain responses (pain threshold, tolerance, and intensity), SOWS, and PSQI scores between DRD2 polymorphisms. Conclusion: The common DRD2 polymorphisms are not associated with pain sensitivity, severity of the opiate withdrawal syndrome, and sleep quality in patients with opioid dependence on MMT. However, this may be unique for Malays. Additional research should focus on investigating these findings in larger samples and different ethnicity.

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INTRODUCTION

Opioid dependence is a chronic disease, which represents a significant problem, with heroin being the most commonly abused opiate.[1,2] In Malaysia, methadone maintenance therapy (MMT) has been used since 2005 as one of the harm reduction programs designed to reduce human immunodeficiency virus (HIV) transmission indirectly by treating patients who have problems with opioid dependence, such as heroin and morphine.[3] Patients on MMT were mainly male of Malay ethnicity.[2-4] Methadone treatment appears to be successful in eliminating withdrawal symptoms, although control of cravings and seeking behaviors cannot be achieved in all patients.[2-3] Patients on MMT have been shown to have methadone-related heightened pain sensitivity and poor sleep quality.[1,5-8]

The outcomes of MMT may be influenced by a combination of environmental, drug-induced, and genetic factors. Genetic association studies in addiction research aim to characterize genetic differences and variation in the processes that contribute to addiction and response to treatment. Attention has been directed to the genetic factors that might influence treatment outcomes of MMT.[9-19] Variation in addiction-related genes (such as dopamine receptor D2 gene, DRD2) due to polymorphisms in the genetic sequence may explain interindividual differences in clinical outcomes of patients on MMT.[9-19]

DRD2 contains polymorphisms that might affect DRD2 expression, density of the receptor, and signal transduction. Polymorphisms in dopamine-related genes have been shown to contribute to interindividual differences in pain sensitivity among healthy subjects and patients, susceptibility to migraine, and response to analgesics.[20-22] The Val96Ala polymorphism in the exon 3, which resides at the second transmembrane domain, was expected to affect the ligand binding pocket of the DRD2 receptor;[23] Pro310Ser and Ser311Cys (dbSNP rs1801028) polymorphisms in the exon 7 at third cytoplasmic domain were expected to affect the interaction with G proteins, and therefore DRD2 signal transduction via cyclic adenosine monophosphate (cAMP) inhibition.[23,24] The -141C Ins/Del (dbSNP rs1799732) polymorphism in the promoter region of the DRD2 was directly related to DRD2 expression.[26] DRD2/ANKK1 TaqI A (also known as DRD2 TaqI A, dbSNP rs1800497, referred to here as TaqI A) polymorphism is thought to be associated with the number of spiperone-binding sites, which may have functional pharmacological relevance. Individuals with TaqI A1 allele seem to have lower striatal DRD2 density compared to those without this allele.[26,27]

DRD2 is one of the dopamine receptors genes that have been studied in relation to opioid dependence.[28,29] The latest meta-analysis by Deng et al.[28] supported the previous meta-analysis study by Chen et al.,[29] which demonstrated that the DRD2/ANKK1 TaqI A (also known as DRD2 TaqI A, dbSNP rs1800497, referred to here as TaqI A) polymorphism played an important role in the development of opioid dependence. In addition, Chen et al.[29] found that the -141C Ins/Del (dbSNP rs1799732) polymorphism was significantly associated with increased risk of opioid dependence. It is possible, therefore, that variations in the DRD2 influence clinical outcomes of patients with opioid dependence on MMT.

There has been little information on the effect of DRD2 polymorphisms on methadone treatment outcomes. There are however studies that investigated the impact of genetic polymorphisms of DRD2 on patient’s dose requirements[14,16] and efficacy[15,17-19] of methadone therapy. However, to the best of our knowledge, data on Ser311Cys (dbSNP rs1801028) and -141C Ins/Del (dbSNP rs1799732) polymorphisms among patients with opioid dependence on MMT are still unavailable for references because information about the relationship between these polymorphisms and methadone outcomes was not reported in the previous studies.[14-19] Dopamine receptor D2 (DRD2) is one of the dopamine receptors that have been studied in relation to pain sensitivity.[30] DRD2 binding in the brain is associated with individual responses to painful stimulation and pain modulatory capacity.[31] Healthy humans with low DRD2 availability in the striatum are associated with a higher cold pain threshold.[31]

Relationship between pain sensitivity, severity of the opiate withdrawal syndrome, and sleep quality with genetic polymorphisms of DRD2 has not been previously reported in patients with opioid dependence on MMT. We aimed in this study to test the hypothesis that DRD2 is associated with interindividual variation in clinical outcomes of patients with opioid dependence on MMT.

KEYWORDS: Dopamine receptor D2, pain sensitivity, severity of the opiate withdrawal syndrome, sleep quality
**Materials and Methods**

**Subjects**

We have reported detailed methods of this study sample previously. Data used in this study were collected as part of the study to evaluate the application of personalized methadone therapy among patients on MMT. In this cross-sectional study, a total of 148 subjects were recruited from 11 outpatient methadone clinics in Kelantan, Malaysia between March and October 2013. In this study, all subjects were stabilized in treatment, defined as having been enrolled in the program for more than 1 month with no change of methadone dosage over the past 1 month.

This 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 at the Ministry of Health, Malaysia (Reference number: NMRR-13-524-16614), and was conducted in accordance with the Declaration of Helsinki.

We recruited only male subjects who were of Malay ethnicity to minimize the possible effects that gender and ethnicity had on pain sensitivity and sleep, and because more than 90% of the patients on MMT in Malaysia are Malay males. All subjects fulfilled inclusion and exclusion criteria and gave informed consent. Inclusion criteria included: (1) subjects aged more than 18 years; (2) free of acute medical, surgical, and psychiatric illness; (3) free of acute or chronic medical, surgical, and psychiatric illness that requires concurrent medical, surgical, or psychiatric therapy; (4) free of regular use of alcohol; (5) free of intoxication; (6) able to understand study protocols and to follow simple study instructions; and (7) willing to sign written informed consent. Alcohol use and intoxication were carefully evaluated during interview and clinical assessment by a physician who assisted the author in this study.

Exclusion criteria were: (1) subjects who were currently taking illicit benzodiazepines, cannabinoids, and barbiturates; (2) subjects with chronic or ongoing acute pain; (3) subjects with a history of analgesic ingestion within 3 days before the cold pressor test (CPT); and (4) subjects with severe cognitive impairment, which may interfere with pain assessments and/or communication.

Urine drug screens for morphine, tetrahydrocannabinol, amphetamines, and benzodiazepines were performed using drugs of abuse rapid test, F.A.C.T.S 4 in 1 Combo Dipcard Rapid Test (MOR/THC/AMP/BZO) (Scientifacts Sdn. Bhd., Selangor, Malaysia) twice in one week at least 3 days apart before the study. Subjects with two consecutive negative urine tests were included in the study. They were instructed to avoid analgesics of any type within 72 h before CPT test day. They have been instructed not to ingest methadone from their MMT clinic on the test morning. Subjects who fulfilled all criteria were interviewed to obtain sociodemographic data and other relevant information.

**Cold pressor test, Subjective Opiate Withdrawal Scale, and Pittsburgh Sleep Quality Index—Malay**

CPT was performed to assess pain threshold, pain tolerance, and pain intensity. Details on CPT methods have been described in our previous articles. The times elapsed between the immersion of hand to pain detection (threshold) and hand withdrawal (tolerance) were quantified in seconds. Immediately after hand withdrawal, subjects were asked to subjectively score their maximal pain intensity using 0–100 visual analog scale (VAS), where zero (0) represented no pain and a hundred (100) represented the worst pain imaginable. The CPT was performed six times over 24 h. The first test was performed approximately 30 min before their morning dose of methadone (0 h), and at 2, 4, 8, 12, and 24 h after the dose.

Patients’ opioid withdrawal symptoms were assessed using the translated and validated Malay version of the Subjective Opiate Withdrawal Scale (SOWS-M). The SOWS-M is a self-administered scale, which contains 16 symptoms. Patients were asked to rate each symptom according to severity. Each item was rated on a four-point Likert scale where zero (0) was “not at all,” one (1) was “a little,” two (2) was “moderately,” three (3) was “quite a bit,” and four (4) was “extremely,” with a total score of 0–64. As this was a patient self-evaluation, the researcher’s role was only to assist the patient to complete the task, not to do it for them or interpret their symptomology. For most patients, the 16-item SOWS-M took less than 10 min to complete. The SOWS-M was administered six times over a 24-h period at 0 h (i.e., immediately [approximately 30 min] before taking their morning dose of methadone), and at 2, 4, 8, 12, and 24 h after the dose intake.

Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI)-Malay. The PSQI-M contains 19 items that are included in scoring. The 19 individual items were 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. 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.
Genotyping of DRD2 polymorphisms

For genotyping, 2 mL of venous blood from each subject was collected. Deoxyribonucleic acid (DNA) was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions with slight modifications. The quantity and quality of the extracted total genomic DNA was determined on the NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, Delaware). Genotyping of eight DRD2 polymorphisms of our research interest (Val96Ala, Leu141Leu, Val154Ile, Pro310Ser, Ser311Cys, TaqI A, -141C Ins/Del, and A-241G) was performed according to previously developed nested allele-specific multiplex polymerase chain reaction (PCR) methods with slight modifications[48] (methods are available on request).

Statistical analysis

Sample size was calculated prior the start of the study based on the Cohen sample size table[49] using medium population effect size (ES) 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 mean values of two groups (under allelic additive model and genotype dominant and recessive model). Allele, genotype, haplotype, and diplotype frequencies were calculated using the program SVS 7.3.1 (GOLDEN HELIX, SNP, and VARIATION SUITE 7) based on an expectation-maximization (EM) algorithm for the following procedures: (a) the calculation of DRD2 alleles and genotypes frequencies; (b) the estimation of heterozygosity in each polymorphism in Hardy–Weinberg proportion; and (c) the estimation of maximum-likelihood haplotype frequency. Assuming a mutant-type allele was a high-risk allele, genotype dominant and recessive model, and haplotypes and diplotypes analysis. Haplotypes and diplotypes with frequencies less than 10% were pooled. The statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS)/Win software (version 24.0, SPSS, Chicago, Illinois). All confidence intervals (CIs) were computed at the 95% level. A probability of <0.05 was considered to be significant for statistical comparison.

RESULTS

Samples demographics

Of the initial 169 subjects who were invited to participate, 21 subjects were excluded from the study (health reasons = 12, scheduling conflicts = 3, and miscellaneous reasons = 6). The average age was 36.86 (standard deviation [SD] = 6.13, range: 25–55) years and the average daily dose of methadone was 72.70 (SD = 28.25, range: 20–160) mg/day with an average duration in MMT of 2.82 (SD = 2.02, range: 0.33–9.00) years.

Genotypic profile

All the DNA samples from the subjects were successfully genotyped. The Val96Ala, Leu141Leu, Val154Ile, and Pro310Ser polymorphisms were not detected in any subject. Allele, genotype, haplotype, and diplotype distributions of presently observed DRD2 polymorphisms are presented in Supplementary Table 1. All the detected polymorphisms were in Hardy–Weinberg equilibrium (HWE) distribution (P > 0.300).

Frequencies of the mutant-type allele for Ser311Cys, TaqI A, -141C Ins/Del, and A-241G polymorphisms were 4.1%, 39.2%, 15.2%, and 16.2%, respectively. There was no subject with 311 Cys/Cys genotype in this study.

When considering loci Ser311Cys, TaqI A, -141C Ins/Del, and A-241G, we observed eight haplotypes in our subjects. Two of them had a frequency higher than 30.0%. The most frequent estimated haplotype was the haplotype no. 1 (CGCA or 311 Ser/Cys, A2/-141C Ins/-241A) (n = 100, 33.8%), followed by the haplotype composed of only wild-type alleles, no. 2 (CACA or 311 Ser/TaqI A1/-141C Ins/-241A) (n = 92, 31.1%). The haplotype composed of only mutant-type alleles (GAAG or 311 Cys/TaqI A1/-141C Del/-241G) was not detected in any subject.

Among the 22 estimated diplotypes, the most common haplotype pair (i.e., diplotype) was diplotype no. 1 (CACA(CGCA)) (n = 27, 18.2%) and diplotype no. 2 (CGCA(CGCA)) (n = 27, 18.2%) followed by diplotype no. 3 (CGAA(CACA)) (n = 13, 8.8%).

Cold pressor test responses, Subjective Opiate Withdrawal Scale, and Pittsburgh Sleep Quality Index–Malay scores among DRD2 polymorphisms

Results showed no significant differences in pain responses (pain threshold, tolerance, and intensity), SOWS, and PSQI scores between presently observed DRD2 polymorphisms (Ser311Cys, TaqI A, -141C Ins/Del, and A-241G) under genotype and allelic additive model, genotype dominant and recessive model, and haplotypes and diplotypes analysis [Tables 1–5].
In this paper, we explored the influence of DRD2 polymorphisms on clinical outcomes of methadone treatment. We were unable to find any association between these specific DRD2 polymorphisms with pain sensitivity, severity of the opiate withdrawal syndrome, and sleep quality during treatment.

In our current study, we genotyped eight DRD2 polymorphisms among 148 opioid-dependent Malay males on MMT. We found that all of the patients were noncarriers of mutant-type alleles for Val96Ala, Leu141Leu, Val154Ile, and Pro310Ser (dbSNP rs1800496) polymorphisms, which were similar to those found among 93 intravenous heroin addicts by Teh et al.[51] and 152 Malay male opioid naive healthy volunteers in our previous study.[40] Genotype distribution for Ser311Cys in our patients was also similar to that observed in the previous study.[40] Among the presently observed mutant-type alleles, the most frequently observed was TaqI A1 (39.2%, 95% CI: 33.6, 44.8), followed by -241G (16.2%, 95% CI: 12.0, 20.4). The frequencies of TaqI A1 and -241G alleles resembled those reported earlier in healthy Malay males (TaqI A1 [38.5%, 95% CI: 33.0, 44.0] and -241G [12.8%, 95% CI: 9.1, 16.6]) and in 596 healthy Japanese males (TaqI A1 [34.1%, 95% CI: 31.4, 36.8] and -241G [11.7%, 95% CI: 8.9, 14.5]).[52] We obtained a -141C Del allelic frequency of 15.2% (95% CI: 11.1, 19.3) and 4.1% (95% CI: 1.8, 6.3) for 311 Cys allele, which were within the range reported for healthy Malay subjects (-141C Del [15.5%, 95% CI: 11.4, 19.5] and 311 Cys [6.6%, 95% CI: 3.8, 9.4]) and healthy Japanese subjects (-141C Del [17.0%, 95% CI: 14.9, 19.2] and 311 Cys [2.8%, 95% CI: 1.4, 4.2]).[52]

We recently evaluated the roles of the cytochrome P450 family 2 subfamily B member 6 (CYP2B6),[10] opioid receptor mu 1 (OPRM1),[12] and P-glycoprotein (P-gp) efflux transporter (ABCB1)[9] gene polymorphisms on pain sensitivity, and found that these genetic factors were significantly associated with pain threshold and pain tolerance among patients with opioid dependence on MMT. The CYP2B6*6 allele has previously been shown to be associated with a lower pain threshold and lower pain tolerance.[10] Interestingly, previous study found that the IVS2+691 CC genotype and AC/AG diplotype of 118A>G and IVS2+691G>C polymorphisms of OPRM1 seem to have opposing roles in pain tolerance.[12] Analysis of relationship between ABCB1 and cold pressor pain response showed that the 2677G>T/A polymorphism and CGC haplotype for the 1236C>T, 2677G>T/A, and 3435C>T polymorphisms of ABCB1 are associated with pain sensitivity among patients with opioid dependence on MMT.[9]

DRD2 has been studied in relation to pain sensitivity.[30] The binding of DRD2 in the brain has been shown to be associated with individual responses to painful stimulation and pain modulatory capacity.[31] In healthy human, low DRD2 availability in the striatum is associated with a higher cold pain threshold.[31] Recently, Jääskeläinen et al.[31] suggested that variation in the DRD2 plays a key role in human pain, thus supporting the hypothesis that 957C>T polymorphism influences dopaminergic pain processing in the striatum, and thereby predicts individual variation of the thermal pain sensitivity.

Despite the important central role of DRD2 in the dopaminergic pain processing, our study did not find any association between the common DRD2 polymorphisms (Ser311Cys, TaqI A, -141C Ins/Del, and A-241G) and cold pressor pain sensitivity, even without the multiple testing corrections. Our findings are in line with our previous study, the only study to examine the role of DRD2 polymorphisms on the interindividual differences in cold pressor pain threshold, pain tolerance, and pain intensity among healthy volunteers.[40] To the best of our knowledge, no prior study has specifically investigated the association of Val96Ala, Leu141Leu, Val154Ile, Pro310Ser, Ser311Cys, TaqI A, -141C Ins/Del, and A-241G polymorphisms and pain sensitivity as a measurement of adverse effects to methadone (i.e., hyperalgesia), thus data of individual polymorphisms are not yet available for comparison.

Does withdrawal symptoms in patients on MMT depend, in part, on polymorphism of the DRD2? In terms of withdrawal symptoms, we were unable to confirm a role of dopaminergic systems in pharmacodynamic effects of methadone. No significant associations were found between DRD2 polymorphisms and the severity of the opiate withdrawal syndrome as rated by 16-item SOWS-M.

Concerning genetic factors for sleep disturbance–related side effects of opioids, thus far, only two studies have been conducted among opioid-addicted individuals during methadone treatment. In both studies (i.e., by Wang et al.[13] and Zahari et al.[14]), the OPRM1 polymorphisms have been shown to be associated with methadone-related side problems. Wang et al.[13] reported a significant association of OPRM1 polymorphisms with insomnia side effects in a Taiwanese MMT cohort. In our previous study, we found that the AC/AG diplotype for the 118A>G and IVS2+691G>C polymorphisms of OPRM1 is
Table 1: Influences of presently observed DRD2 polymorphisms on cold pressor pain threshold

| Polymorphism | N   | Mean# | 95% CI | Lower limit | Upper limit | F stat. (df) | P value* |
|--------------|-----|-------|--------|-------------|-------------|-------------|----------|
| Ser311Cys    |     |       |        |             |             |             |          |
| Genotype (N = 145) | | | | | | | |
| 311 Ser/Ser  | 134 | 25.56 | 20.75 | 30.37 | 0.03 (1) | 0.854 |
| 311 Ser/Cys  | 11  | 27.19 | 10.41 | 43.97 |             |             |          |
| Allele (N = 290) | | | | | | | |
| 311 Ser      | 279 | 25.62 | 22.32 | 28.93 | 0.03 (1) | 0.856 |
| 311 Cys      | 11  | 27.19 | 10.54 | 43.84 |             |             |          |
| Dominant model |     | | | | | | |
| 311 Ser/Ser  | 134 | 25.56 | 20.75 | 30.37 | 0.03 (1) | 0.854 |
| 311 Ser/Cys + 311 Cys/Cys | 11 | 27.19 | 10.41 | 43.97 |             |             |          |
| recessive model |     | | | | | | |
| 311 Ser/Ser + 311 Ser/Cys | 145 | 25.68 | 21.08 | 30.29 |             |             |          |
| 311 Cys/Cys  | 0   | -     | -     | -     |             |             |          |
| Taq1 A       |     |       |        |             |             |             |          |
| Genotype (N = 145) | | | | | | | |
| Taq1 A2/A2   | 57  | 22.99 | 15.62 | 30.36 | 0.61 (2) | 0.547 |
| Taq1 A2/A1   | 62  | 26.28 | 19.21 | 33.34 |             |             |          |
| Taq1 A1/A1   | 26  | 30.18 | 19.27 | 41.08 |             |             |          |
| Allele (N = 290) | | | | | | | |
| Taq1 A2      | 176 | 24.15 | 19.99 | 28.30 | 1.35 (1) | 0.247 |
| Taq1 A1      | 114 | 28.05 | 22.89 | 33.21 |             |             |          |
| Dominant model |     | | | | | | |
| Taq1 A2/A2   | 57  | 22.99 | 15.64 | 30.34 | 0.86 (1) | 0.354 |
| Taq1 A2/A1 + Taq1 A1/A1 | 88 | 27.43 | 21.51 | 33.34 |             |             |          |
| recessive model |     | | | | | | |
| Taq1 A2/A2 + Taq1 A2/A1 | 119 | 24.70 | 19.61 | 29.79 | 0.81 (1) | 0.369 |
| Taq1 A1/A1   | 26  | 30.18 | 19.29 | 41.06 |             |             |          |
| -141C Ins/Del |     | | | | | | |
| Genotype (N = 145) | | | | | | | |
| -141C Ins/Ins | 105 | 25.31 | 19.87 | 30.75 | 0.29 (2) | 0.752 |
| -141C Ins/Del | 36  | 27.73 | 18.44 | 37.02 |             |             |          |
| -141C Del/Del | 4   | 17.19 | -10.68 | 45.06 |             |             |          |
| Allele (N = 290) | | | | | | | |
| -141C Ins    | 246 | 25.66 | 22.14 | 29.18 | 0.00 (1) | 0.974 |
| -141C Del    | 44  | 25.81 | 17.48 | 34.14 |             |             |          |
| Dominant model |     | | | | | | |
| -141C Ins/Ins | 105 | 25.31 | 19.88 | 30.74 | 0.07 (1) | 0.794 |
| -141C Ins/Del + -141C Del/Del | 40 | 26.67 | 17.87 | 35.47 |             |             |          |
| recessive model |     | | | | | | |
| -141C Ins/Ins + -141C Ins/Del | 141 | 25.92 | 21.24 | 30.61 | 0.38 (1) | 0.541 |
| -141C Del/Del | 4   | 17.19 | -10.60 | 44.98 |             |             |          |
| A-241G       |     |       |        |             |             |             |          |
| Genotype (N = 145) | | | | | | | |
| A-241A/A     | 102 | 27.24 | 21.73 | 32.75 | 0.55 (2) | 0.577 |
| A-241A/G     | 38  | 21.64 | 12.61 | 30.66 |             |             |          |
| A-241G/G     | 5   | 24.62 | -0.26 | 49.50 |             |             |          |
| Allele (N = 290) | | | | | | | |
| A-241A       | 242 | 26.36 | 22.82 | 30.91 | 0.86 (1) | 0.355 |
| A-241G       | 48  | 22.26 | 14.30 | 30.22 |             |             |          |
| Dominant model |     | | | | | | |
| A-241A/A     | 102 | 27.24 | 21.75 | 32.73 | 1.06 (1) | 0.304 |
| A-241A/G + A-241G/G | 43 | 21.98 | 13.53 | 30.44 |             |             |          |
| recessive model |     | | | | | | |
| A-241A/A + A-241A/G | 140 | 25.72 | 21.02 | 30.43 | 0.01 (1) | 0.931 |
Table 1: Continue

| Polymorphism     | \( N \) | Mean\# | 95% CI Lower limit | 95% CI Upper limit | \( F \) stat. (\( df \)) \( a \) | \( P \) value\* |
|------------------|---------|--------|-------------------|-------------------|---------------------------|-------------|
| -241G/G          | 5       | 24.62  | -0.27             | 49.51             | 0.84 (4)                  | 0.499       |

Haplotype\( b \) (\( N = 290 \))

| Haplotype | \( N \) | Mean\# | 95% CI Lower limit | 95% CI Upper limit | \( F \) stat. (\( df \)) \( a \) | \( P \) value\* |
|-----------|---------|--------|-------------------|-------------------|---------------------------|-------------|
| CGCA      | 99      | 23.07  | 17.54             | 28.60             | 1.25 (1)                  | 0.265       |
| CACA      | 91      | 29.86  | 24.09             | 35.63             | 3.08 (1)                  | 0.080       |
| CGAA      | 35      | 26.05  | 16.75             | 35.36             | 0.01 (1)                  | 0.921       |
| CGCG      | 33      | 23.83  | 14.25             | 33.41             | 0.15 (1)                  | 0.698       |
| Others\( c \) | 34  | 22.94  | 13.51             | 32.38             | 0.38 (2)                  | 0.685       |
| Not CGCA  | 193     | 26.92  | 22.97             | 30.88             | 3.08 (1)                  | 0.080       |
| Not CACA  | 201     | 23.69  | 19.83             | 27.56             | 0.06 (1)                  | 0.805       |
| Not CGAA  | 35      | 26.05  | 16.75             | 35.36             | 0.01 (1)                  | 0.921       |
| Not CGCG  | 33      | 23.83  | 14.25             | 33.41             | 0.15 (1)                  | 0.698       |

Diplotype (\( N = 145 \))

| Diplotype | \( N \) | Mean\# | 95% CI Lower limit | 95% CI Upper limit | \( F \) stat. (\( df \)) \( a \) | \( P \) value\* |
|-----------|---------|--------|-------------------|-------------------|---------------------------|-------------|
| CACA/CGCA | 26      | 24.38  | 13.49             | 35.27             | 0.46 (2)                  | 0.754       |
| CGCA/CGCA | 27      | 21.90  | 11.22             | 32.59             | 0.06 (1)                  | 0.837       |
| Others\( c \) | 93  | 27.04  | 21.28             | 32.80             | 0.06 (1)                  | 0.805       |
| Not CACA/CGCA | 120 | 25.88  | 20.82             | 30.95             | 0.58 (1)                  | 0.447       |
| Not CGCA/CGCA | 27  | 21.90  | 11.25             | 32.56             | 0.06 (1)                  | 0.805       |
| Not CGGA/CGGA | 119 | 26.46  | 21.38             | 31.53             | 0.06 (1)                  | 0.805       |

\( N \) = number of subject/allele/haplotype/diplototype, CI = confidence interval
\#Pain threshold was recorded as the time elapsed when the subject started to perceive pain after the immersion of their hand, and was quantified in seconds, \( *P \) value is significant at \(<0.05\)
\( a \)Repeated measures ANOVA between group analysis was applied
\( b \)Haplotype patterns were constructed from the four presently observed polymorphisms of \( \text{DRD2} \) (Ser311Cys, TaqI A, -141C Ins/ Del, and \( A-241G \))
\( c \)Haplotype and diplotype with frequency less than 10.0% were pooled under “Others”

associated with better sleep quality among opioid-dependent Malay males on MMT.[11]

Strong biological support for gene-level effects of \( \text{DRD2} \) on sleep and circadian rhythm phenotypes have been reported in the literature.[53] In mice, both \( \text{DRD2} \) presynaptic autoreceptors and post-synaptic heteroreceptors influence dopamine expression levels.[54] and \( \text{DRD2} \) knockout mice show significant differences in wakefulness, non-rapid eye movement sleep, and sleep latency.[55] In human, the first large-scale genetic analysis to show an association between \( \text{DRD2} \) variants and sleep in a multiethnic sample was reported in 2016.[53] This study involved seven Candidate Gene Association Resource (CARe) cohorts of over 25,000 individuals of African, Asian, European, and Hispanic American ancestry. The finding showed that rs17601612 polymorphism of \( \text{DRD2} \) was significantly associated with self-reported, habitual sleep duration. An association was also observed for rs17601612 with polysomnographically determined sleep latency. These findings support a role for \( \text{DRD2} \) in influencing sleep duration. Their work motivates us to examine the association between \( \text{DRD2} \) polymorphisms and clinical outcomes in our Malay males with opioid dependence on MMT. However, our current study did not find any association between \( \text{DRD2} \) polymorphisms and sleep disturbance–related side effects of methadone. The comparison of their study and ours is difficult as the studies are designed differently. However, this may in part be explained by differences in ethnicity of the study populations,[38,39] sample size, the types of \( \text{DRD2} \) polymorphisms investigated in each study, and characteristics of study participants whether the subjects are opioid dependent owing to chronic treatment or are opioid naive.

The findings of our current study have a number of practical implications for future research. Because of the ethnic diversity in Malaysian population, and also because different ethnic groups show differences in pain sensitivity[35-37] and sleep,[38,39] we suggest that
| Polymorphism                  | N   | Mean# | 95% CI        | Lower limit | Upper limit | F stat. (df) | P value* |
|------------------------------|-----|-------|---------------|-------------|-------------|--------------|----------|
| Ser311Cys                    |     |       |               |             |             |              |          |
| Genotype (N = 145)           |     |       |               |             |             |              |          |
| 311 Ser/Ser                  | 134 | 34.47 | 27.90         | 41.04       | 0.04 (1)    | 0.833        |          |
| 311 Ser/Cys                  | 11  | 31.91 | 8.99          | 54.84       |             |              |          |
| Allele (N = 290)             |     |       |               |             |             |              |          |
| 311 Ser                      | 279 | 34.37 | 29.85         | 38.88       | 0.04 (1)    | 0.835        |          |
| 311 Cys                      | 11  | 31.91 | 9.16          | 54.66       |             |              |          |
| Dominant model               |     |       |               |             |             |              |          |
| 311 Ser/Ser                  | 134 | 34.47 | 27.90         | 41.04       | 0.04 (1)    | 0.833        |          |
| 311 Ser/Cys + 311 Cys/Cys    | 11  | 31.91 | 8.99          | 54.84       |             |              |          |
| Recessive model              |     |       |               |             |             |              |          |
| 311 Ser/Ser + 311 Ser/Cys    | 145 | 34.27 | 27.98         | 40.57       |             |              |          |
| 311 Cys/Cys                  | 0   | -     | -             | -           |             |              |          |
| TaqI A                       |     |       |               |             |             |              |          |
| Genotype (N = 145)           |     |       |               |             |             |              |          |
| TaqI A2/A2                   | 57  | 30.70 | 20.62         | 40.78       | 0.42 (2)    | 0.658        |          |
| TaqI A2/A1                   | 62  | 37.04 | 27.38         | 46.70       |             |              |          |
| TaqI A1/A1                   | 26  | 35.52 | 20.59         | 50.44       |             |              |          |
| Allele (N = 290)             |     |       |               |             |             |              |          |
| TaqI A2                      | 176 | 32.93 | 22.75         | 43.11       | 0.55 (1)    | 0.459        |          |
| TaqI A1                      | 114 | 36.34 | 29.28         | 43.41       |             |              |          |
| Dominant model               |     |       |               |             |             |              |          |
| TaqI A2/A2                   | 57  | 30.70 | 20.65         | 40.74       | 0.82 (1)    | 0.368        |          |
| TaqI A2/A1 + TaqI A1/A1      | 88  | 36.59 | 28.51         | 44.67       |             |              |          |
| Recessive model              |     |       |               |             |             |              |          |
| TaqI A2/A2 + TaqI A2/A1      | 119 | 34.00 | 27.03         | 40.97       | 0.03 (1)    | 0.856        |          |
| TaqI A1/A1                   | 26  | 35.52 | 20.60         | 50.43       |             |              |          |
| -141C Ins/Del                |     |       |               |             |             |              |          |
| Genotype (N = 145)           |     |       |               |             |             |              |          |
| -141C Ins/Ins                | 105 | 32.95 | 25.54         | 40.37       | 0.64 (2)    | 0.526        |          |
| -141C Ins/Del                | 36  | 39.60 | 25.97         | 52.26       |             |              |          |
| Allele (N = 290)             |     |       |               |             |             |              |          |
| -141C Ins                    | 246 | 33.92 | 29.11         | 38.73       | 0.13 (1)    | 0.714        |          |
| -141C Del                    | 44  | 36.22 | 24.85         | 47.60       |             |              |          |
| Dominant model               |     |       |               |             |             |              |          |
| -141C Ins/Ins                | 105 | 32.95 | 25.54         | 40.36       | 0.45 (1)    | 0.503        |          |
| -141C Ins/Del + -141C Del/Del | 40  | 37.74 | 25.74         | 49.75       |             |              |          |
| Recessive model              |     |       |               |             |             |              |          |
| -141C Ins/Ins + -141C Del/Del | 141 | 34.65 | 28.25         | 41.04       | 0.49 (1)    | 0.486        |          |
| -141C Del/Del                | 4   | 21.05 | -16.91        | 59.01       |             |              |          |
| A-241G                       |     |       |               |             |             |              |          |
| Genotype (N = 145)           |     |       |               |             |             |              |          |
| -241A/A                      | 102 | 33.73 | 26.18         | 41.28       | 0.10 (2)    | 0.904        |          |
| -241A/G                      | 38  | 36.34 | 23.97         | 48.71       |             |              |          |
| -241G/G                      | 5   | 29.64 | -4.47         | 63.75       |             |              |          |
| Allele (N = 290)             |     |       |               |             |             |              |          |
| -241A                        | 242 | 34.14 | 29.29         | 38.99       | 0.02 (1)    | 0.895        |          |
| -241G                        | 48  | 34.94 | 24.05         | 45.84       |             |              |          |
| Dominant model               |     |       |               |             |             |              |          |
| -241A/A                      | 102 | 33.73 | 26.20         | 41.26       | 0.07 (1)    | 0.794        |          |
| -241A/G + -241G/G            | 43  | 35.56 | 23.97         | 47.16       |             |              |          |
| Recessive model              |     |       |               |             |             |              |          |
| -241A/A + -241A/G            | 140 | 34.44 | 28.01         | 40.86       | 0.08 (1)    | 0.784        |          |
the association of polymorphisms in the DRD2 with cold pressor pain sensitivity and sleep quality also be investigated in other races. Besides DRD2 polymorphisms, it is possible that mutation of dopamine-related genes, which regulate other subtypes of dopamine receptors and dopamine transporters, could explain the large interindividual variation in clinical outcomes of patients on MMT. Thus, future genetic studies of pain and sleep should consider other dopamine-related genes. The clinical impact of DRD2 polymorphism on clinical outcomes of opioid dependent patients on MMT are largely unknown. The results of this study suggested that interindividual differences in clinical outcomes of patients on MMT were not explained by the polymorphisms in the genetic sequence of DRD2. Therefore, identifying DRD2 genotypes for Ser311Cys, TaqI A, -141C Ins/Del, and A-241G polymorphisms among opioid-dependent patients on MMT may have no great impact on the personalized medicine to individualize and optimize methadone substitution treatment, which would be of particular importance during the management of withdrawal symptoms in this patient population.

Limitations of our study deserve consideration. First, the frequencies of 311Cys, -141C Del, and -241G alleles in the study samples were low, thus limiting the interpretation of their influence on clinical outcomes of patients on MMT, although the respective allelic frequencies in our samples were similar to those previously described.[40,51,52] Second, this study recruited only male subjects who were of Malay ethnicity to control for possible confounding effects that gender and ethnicity had on pain and sleep. Unfortunately, we are not able to address potential sex- and ethnic-specific effects of variations of DRD2 on pain and sleep in our study. Therefore, it is not possible for us

### Table 2: Continue

| Polymorphism  | N  | Mean# | 95% CI     | F stat. (df)a | P value* |
|---------------|----|-------|------------|---------------|----------|
| -241G/G       |    |       |            |               |          |
| Haplotypeb (N = 290) |   |       |            |               |          |
| CGCA          | 99 | 31.31 | 23.76-38.86| 0.84 (1)      | 0.360    |
| CACA          | 91 | 36.06 | 28.18-43.94| 0.32 (1)      | 0.570    |
| CGAA          | 35 | 38.09 | 28.01-50.79| 0.42 (1)      | 0.519    |
| CGCG          | 33 | 31.83 | 25.33-44.97| 0.14 (1)      | 0.709    |
| Othersc       | 34 | 35.67 | 18.72-48.46| 0.43 (2)      | 0.652    |
| Not CGCA      | 193| 36.64 | 30.23-41.04| 0.32 (1)      | 0.570    |
| Not CACA      | 201| 33.31 | 28.01-38.62| 0.42 (1)      | 0.519    |
| Not CGAA      | 257| 33.64 | 28.95-38.33| 0.42 (1)      | 0.519    |
| Not CGCG      | 259| 34.47 | 29.80-39.14| 0.43 (2)      | 0.652    |
| Diplotype (N = 145) |   |       |            |               |          |
| CACA/CGCA     | 26 | 33.59 | 18.71-48.46| 0.43 (2)      | 0.652    |
| CGCA/CGCA     | 27 | 28.30 | 13.70-42.90| 0.14 (1)      | 0.709    |
| Othersc       | 93 | 36.04 | 28.17-43.91| 0.01 (1)      | 0.932    |
| Not CACA/CGCA | 120| 34.30 | 27.38-41.22| 0.01 (1)      | 0.932    |
| Not CGCA/CGCA | 27 | 28.30 | 13.75-42.85| 0.78 (1)      | 0.379    |
| Not CGCG/CGCA | 119| 35.50 | 28.57-42.43| 0.78 (1)      | 0.379    |

N = number of subject/allele/haplotype/diplotype, CI = confidence interval

#Pain tolerance was recorded as the time elapsed when the subject withdrew his hand after immersion, and was quantified in seconds, *P value is significant at <0.05

aRepeated measures ANOVA between group analysis was applied

bHaplotype patterns were constructed from the four presently observed polymorphisms of DRD2 (Ser311Cys, TaqI A, -141C Ins/Del, and A-241G)

cHaplotype and diplotype with frequency less than 10.0% were pooled under “Others”
Table 3: Influences of presently observed DRD2 polymorphisms on cold pressor pain intensity

| Polymorphism         | N  | Mean# | 95% CI | F stat. (df) | P value* |
|----------------------|----|-------|--------|--------------|----------|
|                      |    | Lower limit | Upper limit |              |          |
| Ser311Cys Genotype   |    |          |         |              |          |
| (N = 145)            |    |          |         |              |          |
| 311 Ser/Ser          | 134| 64.99   | 62.44  | 67.54        | 1.11 (2) | 0.294   |
| 311 Ser/Cys          | 11 | 69.92   | 61.02  | 78.82        |          |         |
| Allele (N = 290)     |    |          |         |              |          |
| 311 Ser              | 279| 65.19   | 63.43  | 66.95        | 1.07 (1) | 0.302   |
| 311 Cys              | 11 | 69.92   | 61.08  | 78.77        |          |         |
| Dominant model       |    |          |         |              |          |
| 311 Ser/Ser          | 134| 64.99   | 62.44  | 67.54        | 1.11 (1) | 0.294   |
| 311 Ser/Cys + 311 Cys/Cys | 11| 69.92   | 61.02  | 78.82        |          |         |
| Recessive model      |    |          |         |              |          |
| 311 Ser/Ser + 311 Ser/Cys | 145| 65.37   | 62.92  | 67.82        | -        | -        |
| 311 Cys/Cys          | 0  | -       | -      | -            |          |         |
| TaqI A Genotype      |    |          |         |              |          |
| (N = 145)            |    |          |         |              |          |
| TaqI A2/A2           | 57 | 65.52   | 61.59  | 69.45        | 0.28 (2) | 0.753   |
| TaqI A2/A1           | 62 | 64.49   | 60.72  | 68.26        |          |         |
| TaqI A1/A1           | 26 | 67.12   | 61.30  | 72.94        |          |         |
| Allele (N = 290)     |    |          |         |              |          |
| TaqI A2              | 176| 65.16   | 62.94  | 67.38        | 0.09 (1) | 0.769   |
| TaqI A1              | 114| 65.69   | 62.94  | 68.44        |          |         |
| Dominant model       |    |          |         |              |          |
| TaqI A2/A2           | 57 | 65.52   | 61.60  | 69.45        | 0.01 (1) | 0.920   |
| TaqI A2/A1 + TaqI A1/A1 | 88| 65.27   | 62.11  | 68.43        |          |         |
| Recessive model      |    |          |         |              |          |
| TaqI A2/A2 + TaqI A2/A1 | 119| 64.99  | 62.27  | 67.70        | 0.43 (1) | 0.512   |
| TaqI A1/A1           | 26 | 67.12   | 61.31  | 72.92        |          |         |
| -141C Ins/Del Genotype (N = 145) | | | | | |
| -141C Ins/Ins        | 105| 65.86   | 62.96  | 68.75        | 0.21 (2) | 0.814   |
| -141C Ins/Del        | 36 | 64.17   | 59.22  | 69.12        |          |         |
| -141C Del/Del        | 4  | 63.33   | 48.49  | 78.18        |          |         |
| Allele (N = 290)     |    |          |         |              |          |
| -141C Ins            | 246| 65.61   | 63.74  | 67.48        | 0.42 (1) | 0.516   |
| -141C Del            | 44 | 64.02   | 59.59  | 68.45        |          |         |
| Dominant model       |    |          |         |              |          |
| -141C Ins/Ins        | 105| 65.86   | 62.97  | 68.74        | 0.40 (1) | 0.526   |
| -141C Ins/Del + -141C Del/Del | 40| 64.09  | 59.41  | 68.77        |          |         |
| Recessive model      |    |          |         |              |          |
| -141C Ins/Ins + -141C Ins/Del | 141 | 65.43 | 62.93  | 67.92        | 0.08 (1) | 0.783   |
| -141C Del/Del        | 4  | 63.33   | 48.52  | 78.15        |          |         |
| A-241G Genotype      |    |          |         |              |          |
| (N = 145)            |    |          |         |              |          |
| -241A/A              | 102| 65.29   | 62.34  | 68.23        | 0.01 (2) | 0.994   |
| -241A/G              | 38 | 65.59   | 60.76  | 70.41        |          |         |
| -241G/G              | 5  | 65.33   | 52.03  | 78.63        |          |         |
| Allele (N = 290)     |    |          |         |              |          |
| -241A                | 242| 65.33   | 63.44  | 67.22        | 0.01 (1) | 0.933   |
| -241G                | 48 | 65.53   | 61.29  | 69.78        |          |         |
| Dominant model       |    |          |         |              |          |
| -241A/A              | 102| 65.29   | 62.35  | 68.22        | 0.01 (1) | 0.921   |
| -241A/G + -241G/G    | 43 | 65.56   | 61.04  | 70.08        |          |         |
| Recessive model      |    |          |         |              |          |
| -241A/A + -241A/G    | 140| 65.37   | 62.86  | 67.87        | 0.00 (1) | 0.996   |
### Table 3: Continue

| Polymorphism               | N | Mean# | 95% CI | F stat. (df) | P value* |
|----------------------------|---|-------|--------|--------------|----------|
|                            |   | Lower limit | Upper limit |
| -241G/G                    | 5 | 65.33 | 52.08  | 78.58        |
| Haplotypeb (N = 290)       |   |       |        |              |          |
| CGCA                       | 99| 65.45 | 62.50  | 68.41        | 0.41 (4) | 0.800   |
| CACA                       | 91| 65.31 | 62.23  | 68.39        |          |         |
| CGAA                       | 35| 63.72 | 58.75  | 68.69        |          |         |
| CGCG                       | 33| 64.52 | 59.40  | 69.63        |          |         |
| Others c                   | 34| 68.06 | 63.03  | 73.10        |          |         |
| CGCA                       | 99| 65.45 | 62.51  | 68.40        | 0.00 (1) | 0.965   |
| Not CGCA                   | 193| 65.37| 63.26  | 67.48        |          |         |
| CACA                       | 91| 65.31 | 62.24  | 68.39        | 0.00 (1) | 0.947   |
| Not CACA                   | 201| 65.44| 63.37  | 67.51        |          |         |
| CGAA                       | 35| 63.72 | 58.77  | 68.67        | 0.51 (1) | 0.477   |
| Not CGAA                   | 257| 65.63| 63.80  | 67.46        |          |         |
| CGCG                       | 33| 64.52 | 59.41  | 69.62        | 0.13 (1) | 0.717   |
| Not CGCG                   | 259| 65.51| 63.69  | 67.33        |          |         |
| Diplotype (N = 145)        |   |       |        |              |          |
| CACA/CGCA                  | 26| 61.38 | 55.62  | 67.14        | 1.30 (2) | 0.277   |
| CGCA/CGCA                  | 27| 67.59 | 61.94  | 73.25        |          |         |
| Others c                   | 93| 65.89 | 62.84  | 68.93        |          |         |
| CACA/CGCA                  | 26| 61.38 | 55.63  | 67.12        | 2.33 (1) | 0.129   |
| Not CACA/CGCA              | 120| 66.27| 63.60  | 68.95        |          |         |
| CGCA/CGCA                  | 27| 67.59 | 61.92  | 73.26        | 0.72 (1) | 0.399   |
| Not CGCA/CGCA              | 119| 64.90| 62.20  | 67.60        |          |         |

N = number of subject/allele/haplotype/diplotype, CI = confidence interval

#Pain intensity was measured using 0–100 visual analogue scale (VAS), *P value is significant at <0.05

aRepeated measures ANOVA between group analysis was applied

bHaplotype patterns were constructed from the four presently observed polymorphisms of DRD2 (Ser311Cys, TaqI A, -141C Ins/ Del, and A-241G)

cHaplotype and diplotype with frequency less than 10.0% were pooled under “Others”

to extend the conclusion of no association of DRD2 genetic variations on pain and sleep to female subjects or other ethnicities.

**Conclusion**

The DRD2 polymorphisms we studied were not associated with differences in pain sensitivity, severity of the opiate withdrawal syndrome, and sleep quality. However, this may be unique for Malays. Other polymorphisms may be more relevant for Malays. Further investigations in larger numbers, other populations, and polymorphisms are required to confirm these findings.

**Approval of ethical committees**

This study was approved by the Human Research Ethics Committee (HREC), Universiti Sains Malaysia (USM) in Kelantan, Malaysia (Reference number: USM KK/PPP/ JEPeM 253.3 [14]) and the Medical Research and Ethics Committee at the Ministry of Health, Malaysia (Reference number: NMRR-13-524-16614), and was conducted in accordance with the Declaration of Helsinki.

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Table 4: Influences of presently observed *DRD2* polymorphisms on total Subjective Opiate Withdrawal Scale scores

| Polymorphism | N  | Mean# | 95% CI | F stat. (df)* | P value* |
|--------------|----|-------|--------|---------------|---------|
|              |    | Lower limit | Upper limit |
| Ser311Cys    |    |             |         |
| Genotype (N = 144) |    |             |         |
| 311 Ser/Ser | 133 | 3.86 | 3.03 | 4.68 | 0.66 (1) | 0.419 |
| 311 Ser/Cys | 11  | 5.08 | 2.22 | 7.93 |          |        |
| Allele (N = 288) |    |             |         |
| 311 Ser     | 277 | 3.90 | 3.34 | 4.47 | 0.63 (1) | 0.426 |
| 311 Cys     | 11  | 5.08 | 2.24 | 7.92 |          |        |
| Dominant model |    |             |         |
| 311 Ser/Ser | 133 | 3.86 | 3.03 | 4.68 | 0.66 (1) | 0.419 |
| 311 Ser/Cys + 311 Cys/Cys | 11 | 5.08 | 2.22 | 7.93 |          |        |
| Recessive model |    |             |         |
| 311 Ser/Ser + 311 Ser/Cys | 144 | 3.95 | 3.16 | 4.74 |          |        |
| 311 Cys/Cys | 0   | -    | -    | -    |          |        |
| TaqI A      |    |             |         |
| Genotype (N = 144) |    |             |         |
| TaqI A2/A2 | 58  | 4.17 | 2.94 | 5.41 | 1.77 (2) | 0.175 |
| TaqI A2/A1 | 61  | 3.20 | 1.99 | 4.41 |          |        |
| TaqI A1/A1 | 25  | 5.26 | 3.38 | 7.14 |          |        |
| Allele (N = 288) |    |             |         |
| TaqI A2     | 177 | 3.84 | 3.13 | 4.55 | 0.25 (1) | 0.617 |
| TaqI A1     | 111 | 4.13 | 3.23 | 5.02 |          |        |
| Dominant model |    |             |         |
| TaqI A2/A2 | 58  | 4.17 | 2.93 | 5.42 | 0.21 (1) | 0.647 |
| TaqI A2/A1 + TaqI A1/A1 | 86 | 3.80 | 2.77 | 4.82 |          |        |
| Recessive model |    |             |         |
| TaqI A2/A2 + TaqI A2/A1 | 119 | 3.67 | 2.81 | 4.54 | 2.29 (1) | 0.133 |
| TaqI A1/A1 | 25  | 5.26 | 3.37 | 7.15 |          |        |
| -141C Ins/Del |    |             |         |
| Genotype (N = 144) |    |             |         |
| -141C Ins/Ins | 104 | 4.03 | 3.09 | 4.96 | 0.18 (2) | 0.837 |
| -141C Ins/Del | 36  | 3.62 | 2.03 | 5.21 |          |        |
| -141C Del/Del | 4   | 4.92 | 0.15 | 9.68 |          |        |
| Allele (N = 288) |    |             |         |
| -141C Ins   | 244 | 3.97 | 3.36 | 4.57 | 0.02 (1) | 0.889 |
| -141C Del   | 44  | 3.86 | 2.43 | 5.28 |          |        |
| Dominant model |    |             |         |
| -141C Ins/Ins | 104 | 4.03 | 3.09 | 4.96 | 0.10 (1) | 0.758 |
| -141C Ins/Del + -141C Del/Del | 40 | 3.75 | 2.25 | 5.25 |          |        |
| Recessive model |    |             |         |
| -141C Ins/Ins + -141C Ins/Del | 140 | 3.92 | 3.12 | 4.72 | 0.17 (1) | 0.683 |
| -141C Del/Del | 4   | 4.92 | 0.17 | 9.66 |          |        |
| A-241G      |    |             |         |
| Genotype (N = 144) |    |             |         |
| -241A/A     | 101 | 3.74 | 2.80 | 4.68 | 1.37 (2) | 0.258 |
| -241A/G     | 38  | 4.07 | 2.54 | 5.60 |          |        |
| -241G/G     | 5   | 7.33 | 3.11 | 11.56 |          |        |
| Allele (N = 288) |    |             |         |
| -241A       | 240 | 3.79 | 3.18 | 4.40 | 1.62 (1) | 0.204 |
| -241G       | 48  | 4.75 | 3.39 | 6.11 |          |        |
| Dominant model |    |             |         |
| -241A/A     | 101 | 3.74 | 2.79 | 4.68 | 0.67 (1) | 0.415 |
| -241A/G + -241G/G | 43 | 4.45 | 3.00 | 5.90 |          |        |
| Recessive model |    |             |         |
| -241A/A + -241A/G | 139 | 3.83 | 3.03 | 4.63 | 2.62 (1) | 0.108 |
### Table 4: Continue

| Polymorphism       | N  | Mean # | 95% CI | F stat. (df)* | P value* |
|-------------------|----|--------|--------|---------------|----------|
| -241G/G           |    |        |        |               |          |
| Haplotypeb (N = 288) |    |        |        |               |          |
| CGCA              |    |        |        |               |          |
| CACA              |    |        |        |               |          |
| CGAA              |    |        |        |               |          |
| CGCG              |    |        |        |               |          |
| Othersc           |    |        |        |               |          |
| CGCA              |    |        |        |               |          |
| Not CGCA          |    |        |        |               |          |
| CACA              |    |        |        |               |          |
| Not CACA          |    |        |        |               |          |
| CGAA              |    |        |        |               |          |
| Not CGAA          |    |        |        |               |          |
| CGCG              |    |        |        |               |          |
| Not CGCG          |    |        |        |               |          |
| Diplotype (N = 144) |    |        |        |               |          |
| CACA/CGCA         |    |        |        |               |          |
| CGCA/CGCA         |    |        |        |               |          |
| Othersc           |    |        |        |               |          |
| CACA/CGCA         |    |        |        |               |          |
| Not CACA/CGCA     |    |        |        |               |          |
| CGCA/CGCA         |    |        |        |               |          |
| Not CGCA/CGCA     |    |        |        |               |          |

N = number of subject/allele/haplotype/diplotype, CI = confidence interval

#The SOWS-M scores were measured six times over a 24-h period, * P value is significant at <0.05

aRepeated measures ANOVA between group analysis was applied

bHaplotype patterns were constructed from the four presently observed polymorphisms of DRD2 (Ser311Cys, TaqI A, -141C Ins/Del, and A-241G)

cHaplotype and diplotype with frequency less than 10.0% were pooled under “Others”

### Table 5: Influences of presently observed DRD2 polymorphisms on global Pittsburgh Sleep Quality Index scores

| Polymorphism       | N  | Mean | SD  | Test statistic (df) | P value* |
|-------------------|----|------|-----|--------------------|----------|
| Ser311Cys         |    |      |     |                    |          |
| Genotype (N = 148) |    |      |     |                    |          |
| 311 Ser/Ser       | 136| 5.28 | 2.64| -0.68 (146)        | 0.500    |
| 311 Ser/Cys       | 12 | 5.83 | 3.51|                    |          |
| Allele (N = 296)  |    |      |     |                    |          |
| 311 Ser           | 284| 5.30 | 2.68| -0.66 (294)        | 0.507    |
| 311 Cys           | 12 | 5.83 | 3.51|                    |          |
| Dominant model    |    |      |     |                    |          |
| 311 Ser/Ser + 311 Cys/Cys | 136 | 5.28 | 2.64| -0.68 (146)        | 0.500    |
| Recessive model   |    |      |     |                    |          |
| 311 Ser/Ser + 311 Ser/Cys | 148 | 5.32 | 2.71| -                  | -        |
| 311 Cys/Cys       | 0  | -    | -   |                    |          |
| TaqI A            |    |      |     |                    |          |
| Genotype (N = 148) |    |      |     |                    |          |
| TaqI A2/A2        | 58 | 5.60 | 2.81| 0.53 (2, 145)      | 0.590    |
| TaqI A2/A1        | 64 | 5.19 | 2.66|                    |          |
| TaqI A1/A1        | 26 | 5.04 | 2.68|                    |          |
| Allele (N = 296)  |    |      |     |                    |          |
| TaqI A2           | 180| 5.46 | 2.75| 1.04 (294)         | 0.300    |
| TaqI A1           | 116| 5.12 | 2.64|                    |          |
| Polymorphism | N   | Mean | SD  | Test statistic (df) | P value* |
|--------------|-----|------|-----|---------------------|----------|
| Dominant model |     |      |     |                     |          |
| *TaqI A2/A2* | 58  | 5.60 | 2.81| 1.00 (146)*         | 0.317    |
| *TaqI A2/A1 + TaqI A1/A1* | 90  | 5.14 | 2.65|                     |          |
| Recessive model |     |      |     |                     |          |
| *TaqI A2/A2 + TaqI A1/A1* | 122 | 5.39 | 2.73| 0.59 (146)*         | 0.556    |
| *TaqI A1/A1* | 26  | 5.04 | 2.68|                     |          |
| -141C Ins/Del |     |      |     |                     |          |
| Genotype (N = 148) |     |      |     |                     |          |
| *-141C Ins/Ins* | 107 | 5.50 | 2.84| 1.57 (2, 145)*      | 0.211    |
| *-141C Ins/Del* | 37  | 4.70 | 2.04|                     |          |
| *-141C Del/Del* | 4   | 6.50 | 4.20|                     |          |
| Allele (N = 296) |     |      |     |                     |          |
| *-141C Ins* | 251 | 5.38 | 2.74| 0.81 (294)*         | 0.417    |
| *-141C Del* | 45  | 5.02 | 2.51|                     |          |
| Dominant model |     |      |     |                     |          |
| *-141C Ins/Ins* | 107 | 5.50 | 2.84| 1.24 (146)*         | 0.217    |
| *-141C Ins/Del + -141C Del/Del* | 41  | 4.88 | 2.32|                     |          |
| Recessive model |     |      |     |                     |          |
| *-141C Ins/Ins + -141C Ins/Del* | 144 | 5.29 | 2.68| -0.88 (146)*        | 0.382    |
| *-141C Del/Del* | 4   | 6.50 | 4.20|                     |          |
| A-241G |     |      |     |                     |          |
| Genotype (N = 148) |     |      |     |                     |          |
| *-241A/A* | 105 | 5.40 | 2.70| 1.20 (2, 145)*      | 0.303    |
| *-241A/G* | 38  | 4.92 | 2.63|                     |          |
| *-241G/G* | 5   | 6.80 | 3.56|                     |          |
| Allele (N = 296) |     |      |     |                     |          |
| *-241A* | 248 | 5.33 | 2.68| 0.03 (294)*         | 0.974    |
| *-241G* | 48  | 5.31 | 2.87|                     |          |
| Dominant model |     |      |     |                     |          |
| *-241A/A* | 105 | 5.40 | 2.70| 0.53 (146)*         | 0.598    |
| *-241A/G + -241G/G* | 43  | 5.14 | 2.77|                     |          |
| Recessive model |     |      |     |                     |          |
| *-241A/A + -241A/G* | 143 | 5.27 | 2.68| -1.239 (146)*       | 0.217    |
| *-241G/G* | 5   | 6.80 | 3.564|                     |          |
| Haplotypec (N = 296) |     |      |     |                     |          |
| CGCA | 100 | 5.70 | 2.58| 1.21 (4, 291)*      | 0.308    |
| CACA | 92  | 4.99 | 2.72|                     |          |
| CGAA | 35  | 5.00 | 2.65|                     |          |
| CGCG | 33  | 5.06 | 3.05|                     |          |
| Othersd | 36  | 5.69 | 2.71|                     |          |
| CGCA | 100 | 5.70 | 2.58| 1.71 (294)*         | 0.088    |
| Not CGCA | 196 | 5.13 | 2.76|                     |          |
| CACA | 92  | 4.99 | 2.72| -1.43 (294)*        | 0.153    |
| Not CACA | 204 | 5.48 | 2.70|                     |          |
| CGAA | 35  | 5.00 | 2.65| -0.75 (294)*        | 0.452    |
| Not CGAA | 261 | 5.37 | 2.72|                     |          |
| CGCG | 33  | 5.06 | 3.05| 0.59 (294)*         | 0.554    |
| Not CGCG | 263 | 5.36 | 2.67|                     |          |
| Diplotype (N = 148) |     |      |     |                     |          |
| CACA/CGCA | 27  | 5.56 | 2.76| 1.80 (2, 145)*      | 0.169    |
| CGCA/CGCA | 27  | 6.11 | 2.59|                     |          |
| Othersd | 94  | 5.03 | 2.71|                     |          |
Table 5: Continue

| Polymorphism          | N  | Mean | SD  | Test statistic (df) | P value* |
|-----------------------|----|------|-----|---------------------|---------|
| CACA/CGCA             | 27 | 5.56 | 2.76| 0.49 (146)*         | 0.626   |
| Not CACA/CGCA         | 121| 5.27 | 2.71|                     |         |
| CGCA/CGCA             | 27 | 6.11 | 2.59| 1.68 (146)*         | 0.096   |
| Not CGCA/CGCA         | 121| 5.15 | 2.72|                     |         |

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

*P value is significant at <0.05

†statistic using independent t test

‡F statistic using one-way ANOVA test

Haplotype patterns were constructed from the four presently observed polymorphisms of DRD2 (Ser311Cys, TaqI A, -141C Ins/ Del, and A-241G)

Haplotype and diplotype with frequency less than 10.0% were pooled under “Others”

Conflicts of interest

There are no conflicts of interest.

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### Supplementary Table 1: Allele, genotype, haplotype, and diplotype distributions of presently observed DRD2 polymorphisms

| Polymorphism | Genotype/allele | N  | Frequency (%) | 95% CI | HWE P value* |
|--------------|-----------------|----|---------------|--------|--------------|
|              |                 |    |               | Lower limit | Upper limit |
| Ser311Cys<sup>a</sup> | 311 Ser/Ser     | 136| 91.9          | 87.5   | 96.3         | 1.000         |
|              | 311 Ser/Cys     | 12 | 8.1           | 3.7    | 12.5         |               |
|              | 311 Cys/Cys     | 0  | 0.0           | 0.0    | 0.0          |               |
| Allele (N = 296) | 311 Ser    | 284| 95.9          | 93.7   | 98.2         |               |
|              | 311 Cys        | 12 | 4.1           | 1.8    | 6.3          |               |
| Dominant model | 311 Ser/Ser | 136| 91.9          | 87.5   | 96.3         |               |
|              | 311 Ser/Cys + 311 Cys/Cys | 12| 8.1          | 3.7    | 12.5         |               |
| Recessive model | 311 Ser/Ser + 311 Ser/Cys | 148| 100.0        | 100.0  | 100.0        |               |
|              | 311 Cys/Cys    | 0  | 0.0           | 0.0    | 0.0          |               |
| TaqI<sup>b</sup> | Genotype (N = 148) |  |               |         |              |
|              | Taql A2/A2     | 58 | 39.2          | 31.3   | 47.1         | 0.300         |
|              | Taql A2/A1     | 64 | 43.2          | 35.3   | 51.2         |               |
|              | Taql A1/A1     | 26 | 17.6          | 11.4   | 23.7         |               |
| Allele (N = 296) | Taql A2    | 180| 60.8          | 55.2   | 66.4         |               |
|              | Taql A1        | 116| 39.2          | 33.6   | 44.8         |               |
| Dominant model | Taql A2/A2 | 58 | 39.2          | 31.3   | 47.1         |               |
|              | Taql A2/A1 + Taql A1/A1 | 90| 60.8         | 52.9   | 68.7         |               |
| Recessive model | Taql A2/A2 + Taql A2/A1 | 122| 82.4        | 76.3   | 88.6         |               |
|              | Taql A1/A1     | 26 | 17.6          | 11.4   | 23.7         |               |
| -141C Ins/Del<sup>c</sup> | Genotype (N = 148) |  |               |         |              |
|              | -141C Ins/Ins  | 107| 72.3          | 65.1   | 79.5         | 0.748         |
|              | -141C Ins/Del  | 37 | 25.0          | 18.0   | 32.0         |               |
|              | -141C Del/Del  | 4  | 2.7           | 0.1    | 5.3          |               |
| Allele (N = 296) | -141C Ins    | 251| 84.8          | 80.7   | 88.9         |               |
|              | -141C Del      | 45 | 15.2          | 11.1   | 19.3         |               |
| Dominant model | -141C Ins/Ins | 107| 72.3          | 65.1   | 79.5         |               |
|              | -141C Ins/Del + -141C Del/Del | 41| 27.7        | 20.5   | 34.9         |               |
| Recessive model | -141C Ins/Ins + -141C Ins/Del | 144| 97.3       | 94.7   | 99.9         |               |
|              | -141C Del/Del  | 4  | 2.7           | 0.1    | 5.3          |               |
| A-241G<sup>d</sup> | Genotype (N = 148) |  |               |         |              |
|              | -241A/A        | 105| 70.9          | 63.6   | 78.3         | 0.542         |
|              | -241A/G        | 38 | 25.7          | 18.6   | 32.7         |               |
|              | -241G/G        | 5  | 3.4           | 0.5    | 6.3          |               |
| Allele (N = 296) | -241A    | 248| 83.8          | 79.6   | 88.0         |               |
|              | -241G         | 48 | 16.2          | 12.0   | 20.4         |               |
| Dominant model | -241A/A | 105| 70.9          | 63.6   | 78.3         |               |
|              | -241A/G       | 43 | 29.1          | 21.7   | 36.4         |               |
| Recessive model | -241A/A + -241A/G | 143| 96.6        | 93.7   | 99.5         |               |
|              | -241G/G       | 5  | 3.4           | 0.5    | 6.3          |               |
| Haplotype<sup>e</sup> (N = 296) |  |               |         |              |
|              | CGCA           | 100| 33.8          | 28.4   | 39.2         |               |
|              | CACA           | 92 | 31.1          | 25.8   | 36.4         |               |
|              | CGAA           | 35 | 11.8          | 8.1    | 15.5         |               |
|              | CGCG           | 33 | 11.1          | 7.6    | 14.7         |               |
|              | CACG           | 14 | 4.7           | 2.3    | 7.1          |               |
|              | GGCA           | 11 | 3.7           | 1.6    | 5.9          |               |
|              | CAAA           | 10 | 3.4           | 1.3    | 5.4          |               |
|              | GGCG           | 1  | 0.3           | 0.0    | 1.0          |               |
| Diplotype (N = 148) |  |               |         |              |
|              | CACA/CGCA     | 27 | 18.2          | 13.8   | 22.6         |               |
|              | CGCA/CGCA     | 27 | 18.2          | 13.8   | 22.6         |               |
|              | CGAA/CACA     | 13 | 8.8           | 5.6    | 12.0         |               |
|              | CACA/CACA     | 12 | 8.1           | 5.0    | 11.2         |               |
| Polymorphism | Genotype/allele | N  | Frequency (%) | 95% CI | HWE P value* |
|--------------|----------------|----|---------------|--------|--------------|
|              |                |    |               | Lower limit | Upper limit  |
| CGCA/CGCG    | 11             | 7.4 | 4.4           | 10.4               |
| CACA/CGCG    | 10             | 6.8 | 3.9           | 9.6               |
| CAAA/CACA    | 7              | 4.7 | 2.3           | 7.1               |
| CACA/CACG    | 7              | 4.7 | 2.3           | 7.1               |
| CGAA/CGCA    | 7              | 4.7 | 2.3           | 7.1               |
| CGAA/CGCG    | 5              | 3.4 | 1.3           | 5.4               |
| CACA/GGCA    | 4              | 2.7 | 0.9           | 4.6               |
| CACG/CGCG    | 3              | 2.0 | 0.4           | 3.6               |
| CGAA/CGAA    | 3              | 2.0 | 0.4           | 3.6               |
| CAAA/GGCA    | 2              | 1.4 | 0.0           | 2.7               |
| CGAA/CACG    | 2              | 1.4 | 0.0           | 2.7               |
| GGCA/CACG    | 2              | 1.4 | 0.0           | 2.7               |
| CAAA/GGCA    | 1              | 0.7 | 0.0           | 1.6               |
| CGAA/GGCA    | 1              | 0.7 | 0.0           | 1.6               |
| CGCA/GGCA    | 1              | 0.7 | 0.0           | 1.6               |
| CGCG/CGCG    | 1              | 0.7 | 0.0           | 1.6               |
| GGCA/CGCG    | 1              | 0.7 | 0.0           | 1.6               |
| CAAA/CGAA    | 1              | 0.7 | 0.0           | 1.6               |
| CGAA/GGCA    | 1              | 0.7 | 0.0           | 1.6               |
| CGCG/CGCG    | 1              | 0.7 | 0.0           | 1.6               |
| CGCA/CGCG    | 1              | 0.7 | 0.0           | 1.6               |

N = number of subject/allele/haplotype/diplotype, CI = confidence interval, HWE = Hardy-Weinberg equilibrium

*Fisher’s exact test evaluates the probability of genotype counts that are equally or less likely than the observed counts under HWE for biallelic locus

1. dbSNP rs1801028 (substitution of cysteine for serine at codon 311, nucleotide change at 20229C>G, accession number: AF050737)
2. dbSNP rs1800497 (alteration of a TaqI restriction site at 10 541 kb downstream of DRD2 stop codon (exon 8), nucleotide change at 32806C>T, accession number: AF050737)
3. dbSNP rs1799732 (single base pair cytosine insertion/deletion at position -141, nucleotide change at 6191 C>–, accession number: AF148806)
4. dbSNP rs1799978 (substitution of guanine for adenosine at -241, nucleotide change at 6091A>G, accession number: AF148806)

5. Haplotype patterns were constructed from the four presently observed polymorphisms of DRD2 (Ser311Cys, TaqI A, -141C Ins/Del, and A-241G)