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Functional Genetic Polymorphisms in *CYP2C19* Gene in Relation to Cardiac Side Effects and Treatment Dose in a Methadone Maintenance Cohort

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Abstract

Methadone maintenance therapy is an established treatment for heroin dependence. This study tested the influence of functional genetic polymorphisms in *CYP2C19* gene encoding a CYP450 enzyme that contributes to methadone metabolism on treatment dose, plasma concentration, and side effects of methadone. Two single nucleotide polymorphisms (SNPs), rs4986893 (exon 4) and rs4244285 (exon 5), were selected and genotyped in 366 patients receiving methadone maintenance therapy in Taiwan. The steady-state plasma concentrations of both methadone and its EDDP metabolite enantiomers were measured. SNP rs4244285 allele was significantly associated with the corrected QT interval (QTc) change in the electrocardiogram ($p = 0.021$), and the Treatment Emergent Symptom Scale (TESS) total score ($p = 0.021$) in patients who continued using heroin, as demonstrated with a positive urine opiate test. Using the gene dose (GD) models where the *CYP2C19* SNPs were clustered into poor (0 GD) versus intermediate (1 GD) and extensive (2 GD) metabolizers, we found that the extensive metabolizers required a higher dose of methadone ($p = 0.035$), and showed a lower plasma *R*-methadone/methadone dose ratio ($p = 0.007$) in urine opiate test negative patients, as well as a greater QTc change ($p = 0.008$) and higher total scores of TESS ($p = 0.018$) in urine opiate test positive patients, than poor metabolizers. These results in a large study sample from Taiwan suggest that the gene dose of *CYP2C19* may potentially serve as an indicator for the plasma *R*-methadone/methadone dose ratio and cardiac side effect in patients receiving methadone maintenance therapy. Further studies of pharmacogenetic variation in methadone pharmacokinetics and pharmacodynamics are warranted in different world populations.

Introduction

Methadone, a synthetic opioid used as an analgesic agent (Leimanis et al., 2012), is well known mostly as a maintenance medication to prevent withdrawal symptoms and to improve quality of life for heroin-dependent patients (Blanken et al., 2012; Wang et al., 2012). One of the adverse reactions following methadone treatment is the prolongation of the cardiac QT interval determined by electrocardiogram (ECG) (Fonseca et al., 2009). Therefore, patients who receive a

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high dose of methadone are recommended to be monitored for the QT interval (Byrne and Stimmel, 2007). It has been suggested that the different enantiomers of methadone may contribute to the manifestation of its multiple effects (Hutchinson and Somogyi, 2004). The *R*-form enantiomer of methadone has been found to have a more efficacious analgesic effect (Kristensen et al., 1996), but a lower cardiac toxic effect (Ansermot et al., 2010) than the *S*-form, whereas *S*-methadone is associated with an unsatisfactory efficacy in methadone maintenance therapy (MMT) for opioid dependence (Elkader et al., 2009). These observations indicate that the differences in the pharmacological effects of methadone are due to the respective enantiomers in the plasma.

Methadone is extensively metabolized in the liver through specific isoforms of the CYP enzyme system (Gerber et al., 2004). It has been reported that specific isoforms involving the metabolism of methadone include CYP2B6, CYP3A4, and to a minor effect CYP2C19 and CYP2D6 (Crettol et al., 2005; Gerber et al., 2004; Wang and DeVane, 2003). In addition, it appears that the two methadone enantiomers are preferentially metabolized by specific CYP isozymes, for example, the *R*-form by CYP2C19, and the *S*-form by CYP2B6 (Chang et al., 2011; Gerber et al., 2004; Totah et al., 2008). Besides the enzymatic contribution, polymorphisms in the gene encoding a transmembrane efflux transporter may contribute slightly to the individual variability of methadone kinetics (Crettol et al., 2006). In clinical practice, methadone is usually administered to patients in a racemic mixture that contains an equal amount of the *R*- and *S*-enantiomer (Eap et al., 2000). In our previous study, we established a system for measuring the plasma levels of both enantiomers of methadone and its metabolite 2-ethylidene-1,5-dimethyl- 3,3-diphenylpyrrolidine (EDDP) (Wang et al., 2010). We hypothesize that the respective enantiomer and CYP isomer preference in methadone metabolism may influence the steady-state plasma concentrations of methadone, and that the variations in the genes encoding the CYP enzymes may be associated with the plasma concentrations of methadone and side effects. Analysis of these genetic variants in the genes encoding the metabolic enzymes may provide further insights into the mechanisms through which methadone exerts its pharmacological effects.

CYP2C is a subfamily of the human CYP metabolic enzymes in the liver. It is estimated that CYP2C is responsible for about 20% of the metabolism of all clinically used medications (Goldstein, 2001). CYP2C19 comprises 16% of the CYP2C subfamily (Gerbal-Chaloin et al., 2001). The *CYP2C19* gene was mapped to chromosome 10q24.1-24.3 in human. It contains nine exons (MIM ID *124020). This enzyme catalyzes the oxidation of several clinically important medications including proton pump inhibitors (PPIs) (Dickson and Stuart, 2003), and some endogenous hormones (Ingelman-Sundberg et al., 2007). Among all genetic variants that have been characterized in the *CYP2C19* gene, *CYP2C19* *2 (681G > A of rs4244285 which causes a splicing defect) and *CYP2C19* *3 (636G > A of rs4986893, which causes a premature stop codon) (Ferguson et al., 1998) are the most well studied functional SNPs. The minor allele frequencies (MAF) for *2 SNP are 0.15 in Caucasians and 0.256 in Chinese. respectively (NCBI, 2011), whereas the MAF for *3 SNP are 0.5 in Caucasians and 0.533 in Chinese. respectively (NCBI, 2011). *CYP2C19* *2 and *CYP2C19* *3 have been reported to be associated with a poor metabolizer phenotype in both Caucasian and Asian populations (De Morais et al., 1994; Mizutani, 2003). These two major SNPs were therefore selected for the current study.

In this study, we tested whether the two major SNPs in *CYP2C19* are associated with the plasma concentrations of methadone and its enantiomers, the methadone treatment dosage, and the side effects in a Taiwanese MMT cohort.

Material and Methods

Subjects

This study was approved by the institutional review boards of the National Health Research Institutes (Zhunan, Taiwan) and all six participating hospitals. Written informed consents were obtained from all participants. The project has also been registered with the National Institutes of Health Clinical Trial (NIH, 2011). 366 heroin-dependent patients undergoing methadone maintenance treatment as outpatients were recruited into the study. The inclusion criteria included an age of 18 years or above, receiving MMT for at least 3 months with regular attendance in the past 7 days, and a methadone dosage adjustment of not more than 10 mg in the past 7 days. Exclusion criteria included co-morbidity with physical and mental disorders that require immediate treatment or pregnancy.

Clinical assessments

Demographics, medical co-morbidity, substance use history, and methadone treatment course, including the dose, treatment duration, and treatment compliance over the previous week, were obtained from the medical records. The Treatment Emergent Symptom Scale (TESS) (Guy, 1976), an interviewer-administrated instrument, was used to assess adverse events related to methadone treatment. All participating hospitals used the same protocol and same standard in the interpretation of data.

ECG assessments

The electrocardiogram (ECG) measure was performed in each participating hospital according to the regular standard operation procedure (SOP). The ECGs were visually inspected by an experienced cardiologist who was blinded to the study. The cardiologist excluded those signals with technical errors or with inadequate quality for further analysis. ECG assessments were performed using a standard 12-lead recording apparatus. The baseline ECG, measured before the subjects entered the methadone treatment program, was obtained from medical records. The current ECG was assessed before the intake of the last dose of methadone on the study day. The QT interval, corrected for heart rate according to the Bazzet formula (QTc), was used for subsequent analysis (Bazett, 1920). The QTc change represents the difference between baseline and current QTc intervals for patients with a complete set of baseline and current ECG measurement data.

Urine drug test

Urine specimens were collected prior to methadone intake. The opiate screen test in the urine was assessed via a kinetic interaction of microparticles (KIMS) method with an Integra 800 device (Roche Diagnostics, Basel, Switzerland). Urine opiate test was used as a surrogate measurement of the methadone treatment outcome in the present study.

Analysis of methadone and its metabolites in plasma

Twelve mL of whole blood samples were collected with ethylenediaminetetraacetic acid (EDTA) as anticoagulant at 24 ± 2 h after the last methadone intake, the time at which the plasma concentration of methadone is likely the lowest. The plasma was obtained from centrifugation of the whole blood at 2000 *g* for 20 min. Plasma concentrations of the enantiomers of both methadone and its metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), were measured using high-performance liquid chromatography (HPLC) with UV-detection at 210 nm wavelength (Wang et al., 2010). Methadone, EDDP, and a non-opioid amitriptyline as an internal standard (40 ng) were extracted from the plasma samples using a C18-E 100 mg/mL capacity Strata solid-phase extraction column (Phenomenex, Torrance, CA). Following conditioning of the column on a vacuum manifold (Waters, Milford, MA), 800 μL aliquots of each plasma sample and 40 ng of the amitriptyline internal standard were added. The column was then washed and the retained compounds were eluted with 1 mL of ammonium phosphate (monobasic)/methanol (0.01g/100 mL). The collected eluent was then evaporated and the remaining residue was dissolved in 100 μL of the mobile phase. A total sample volume of 50 μL was then injected into the chromatograph.

CYP2C19 SNP selection and genotyping

Genomic DNA for each patient was extracted from the buffy coat of 6 mL whole blood lymphocyte pellets using the Puregene® Blood kit C (QIAGEN Sciences; Maryland, USA). The genomic DNA was used for genotyping allelic polymorphisms in rs4244285 (*CYP2C19*2*) and rs4986893 (*CYP2C19*3*).

The genotypes of the two SNPs were identified using matrix-assisted laser desorption/ ionization-time of flight mass spectrometry (MALDI-TOF MS) (Rodi et al., 2002). Primers and probes flanking the SNPs were designed using SpectroDESIGNER software (Sequenom, San Diego, CA). DNA fragments (100–300 bp) encompassing each SNP site were amplified by PCR (GeneAmp 9700 thermocycler, Applied Biosystems, Foster City, CA) in accordance with the manufacturer's instructions.

After removal of the un-incorporated dNTPs and inactivation of the shrimp alkaline phosphatase (SAP) from the PCR reaction, primer extension was performed via the addition of the appropriate probe, Thermo Sequenase (Amersham Pharmacia, Piscataway, NJ) and a dNTP mixture. The reaction conditions were 45 cycles of denaturation at 94°C for 5 sec, annealing at 56°C for 5 sec, and extension at 72°C for 5 sec. The various extension products were differentiated by MALDI-TOF analysis. This genotyping method has been applied in a broad variety of clinical applications because of its accuracy of SNP detection, sufficient sensitivity to score SNPs from small amounts of template, flexibility of the procedure, and cost-effectiveness (Tost and Gut, 2005).

Statistics

All statistical analyses were conducted using SAS software, Version 9.2 (SAS Institute, Inc., Cary, NC). The urine opiate test and plasma methadone concentration were analyzed by nonparametric Mann-Whitney U test. Association analyses between SNPs in *CYP2C19* (genotype or allele type or gene dose, GD) and methadone dose, QTc change, sum of TESS scores, the ratios of *R*-and *S*-methadone/dose, and the ratios of *R*-and *S*-EDDP/dose were calculated using permutation test with the MULTTEST procedure. This test was applied for correction of multiple testing and for adjustment of p values corresponding to a nominal type I error of 5%. The correlations between the GD and methadone dose, change in QTc interval, sum of TESS scores, the ratios of *R*-and *S*-methadone/dose, *R*-and *S*-EDDP/dose, *R*-and *S*-EDDP/*R*,*S*-methadone, *R*-EDDP/*S*-EDDP, and *R*-methadone/*S*-methadone were calculated by the Kendall's coefficient of rank correlation for nonparametric analyses and for further comparisons between subgroups. The Kendall's correlation coefficient is used to evaluate the association for a directionality null hypothesis. The Hardy-Weinberg equilibrium tests were performed using HAPLOVIEW version 4.1 (Barrett et al., 2005). The statistical significant level was set for p values smaller than 0.05.

Results

Demographics and clinical characteristics

The clinical and genotyping data from all 366 MMT subjects in the cohort were included for analyses. Males were predominant ($n = 297$, 81.1%) in this cohort. The average age was 38.0 years, and most patients smoked cigarettes. The average dose of methadone was 54.7 ± 28.1 mg/day with an average treatment duration for 65 weeks. 51% were tested positive for the urine opiate test. Constipation (67.8%), sedation (47.0%), changes in libido (30.3%), dry mouth (27.6%), impaired mentation (21.6%), excessive sweating (19.4%), insomnia (18.3%), and fatigue (17.8%) were the eight most common methadone-related adverse events. Other medications taken by patients were listed in Supplementary Table S1 (supplementary material is available online at www.liebertpub.com/omi). The baseline and current mean QTc intervals were 402.8 ± 27.1 ms and 415.6 ± 23.4 ms ($n = 185$) respectively. Complete data for both baseline and current ECG assessments were available in 185 subjects with an average QTc change as 12.7 ± 25.9 (ranging from −64 to 116) ms. The average QTc change of the 92 subjects who were tested positive for the urine opiate test among the 185 subjects was 15.9 ± 27.4 ms.

Methadone plasma concentration and treatment outcome

The average plasma concentrations of methadone and EDDP were 193.07 ± 121.76, 142.20 ± 99.09 ng/mL for *R*- and *S*-methadone, and 13.70 ± 15.13, 14.64 ± 12.98 ng/mL for *R*- and *S*-EDDP, respectively. Subjects with a favorable outcome, defined as a negative urine opiate test, had a higher concentration-to-dosage ratio (C/D ratio) of both *R*- (4.03 ± 1.82 ng/mL-mg vs. 3.70 ± 2.71 ng/mL-mg) (Mann-Whitney U test $p = 0.001$) and *S*-methadone (2.98 ± 1.66 ng/mL-mg vs. 2.58 ± 1.45 ng/mL-mg) (Mann-Whitney U test, $p = 0.012$).

Single locus of CYP2C19 and GD model

rs4986893 (located at exon 4) and rs4244285 (exon 5) were in Hardy-Weinberg equilibrium with minor allele frequencies of 0.042 and 0.32 respectively. Table 1 describes the frequencies, effects, and alternations in enzyme activity of the rs4986893–rs4244285 allelic combinations. In addition, the GD of the

Table 1. *CYP*2C19 Gene Dosage in Study Population

Gene dose	N	Genotype		Allele combination	Predicted phenotype
		rs4244285	*rs4986893*		
0	39	AA	GG	*2/*2	PM[a]
	14	GA	GA	*2/*3	PM[a]
	1	GG	AA	*3/*3	PM[a]
1	139	GA	GG	*1/*2	IM[b]
	15	GG	GA	*1/*3	IM[b]
2	158	GG	GG	*1/*1	EM[c]

[a]PM, poor metabolizer; [b]IM, intermediate metabolizer; [c]EM, extensive metabolizer.

*1 (Wild type), G-G (rs4244285–rs4986893), haplotype frequency 0.642; *2 (Splice-site mutation of inactive CYP2C19; 681G > A), A-G (rs4244285-rs4986893), haplotype frequency 0.315; *3 (W212X of inactive CYP2C19; 636G > A): G-A (rs4244285–rs4986893), haplotype frequency 0.042.

allelic combinations was determined for all patients. Subjects were then further subgrouped as poor, intermediate, or extensive metabolizers, based on the GD results. The poor metabolizers included 0 GD (allelic combinations of *2/*2, *2/*3 and *3/*3), the intermediate metabolizers included 1 GD (allelic combinations of *1/*2 and *1/*3) and the extensive metabolizers of 2 GD (allelic combinations of *1/*1).

CYP2C19 is associated with methadone dose and plasma concentrations of R-methadone and metabolite in urine opiate test negative patients

In urine opiate test negative patients, rs4244285 allele type and genotype (permutation, $p = 0.013$ and 0.035) showed significant associations with *R*-methadone/dose ratio in single locus association analyses (Table 2). The *R*-methadone/dose ratio was 3.85 ± 1.81 in the extensive metabolizer G allele carriers, which was significantly lower than that in the A allele carriers (4.40 ± 1.79). The allele type and genotype (data not shown) of rs4986893 were not significantly associated with the dose-corrected plasma concentrations of methadone enantiomers or its metabolites.

The *CYP2C19* GD was significantly associated with the methadone daily dose, and the dose-corrected plasma concentrations of both *R*-methadone and *R*-EDDP. The GD was significantly associated with the methadone dose ($p = 0.046$ or 0.035 after removing 10 patients who had taken medications that would affect the CYP2C19 activities). The methadone daily doses of both the extensive metabolizers of 2 GD (58.78 ± 32.69 mg/d, $p = 0.004$) and intermediate metabolizers of 1 GD (57.64 ± 28.69 mg/d, $p = 0.001$) were significantly higher than that of the poor metabolizers of 0 GD (40.45 ± 22.17 mg/d). Furthermore, the poor metabolizers (0 GD) had higher plasma concentrations of both dose-corrected plasma concentrations of *R*-methadone ($p = 0.002$) and *R*-EDDP ($p = 0.03$) than extensive metabolizers of 2 GD in all subjects.

The ratios of *R*-EDDP/*S*-EDDP, *R*-methadone/*S*-methadone, *R*-EDDP/*R*-methadone and *S*-EDDP/*S*-methadone were not significantly associated with the gene dose or SNP rs4986893 in either urine opiate test negative patients (Supplementary Table S2) or in urine opiate test positive patients who showed significant QTc change (Supplementary Table S3).

Table 2. Association Analyses of *CYP*2C19 Genetic Variants and the Methadone Dose, Ratios of Plasma Methadone or Metabolite/Dose, and Side Effect Total Scores in Urine Opiate Test Negative Patients

SNP/Gene dose	Allele/ Gene dose	Methadone dosage (mg/day)			Sum of Tess			R-Methadone/dosage ratio			S-Methadone/dosage ratio			R-EDDP/dosage ratio			S-EDDP/dosage ratio		
		N	Mean ± SD	p value	N	Mean ± SD	p value	N	Mean ± SD	p value	N	Mean ± SD	p value	N	Mean ± SD	p value	N	Mean ± SD	p value
rs4244285	A	116	51.17 ± 28.29	0.07 (0.05)	116	5.62 ± 5.99	0.64 (0.41)	116	4.40 ± 1.79	0.006 (0.013)	116	3.09 ± 1.66	0.39 (0.40)	113	0.40 ± 0.64	0.10 (0.15)	113	0.31 ± 0.29	0.72 (0.57)
	G	240	57.32 ± 30.77		240	5.92 ± 5.24		240	3.85 ± 1.81		240	2.93 ± 1.66		233	0.30 ± 0.47		239	0.34 ± 0.68	
rs4986893	A	16	42.19 ± 16.43	0.08 (0.06)	16	5.63 ± 4.51	0.89 (0.94)	16	3.84 ± 1.66	0.67 (0.80)	16	2.92 ± 1.49	0.89 (0.84)	16	0.32 ± 0.20	0.92 (0.94)	16	0.40 ± 0.39	0.51 (0.29)
	G	340	55.94 ± 30.45		340	5.83 ± 5.54		340	4.04 ± 1.83		340	2.98 ± 1.67		330	0.33 ± 0.55		336	0.33 ± 0.59	
Gene Dose	0	29	40.45 ± 22.17	0.046 (0.035)	29	5.34 ± 5.34	0.22 (0.15)	29	4.36 ± 1.69	0.003 (0.007)	29	3.14 ± 1.71	0.35 (0.37)	28	0.33 ± 0.21	0.015 (0.035)	28	0.33 ± 0.17	0.042 (0.06)
	1	74	57.64 ± 28.69		74	5.84 ± 6.23		74	4.31 ± 1.87		74	3.01 ± 1.60		73	0.43 ± 0.78		73	0.32 ± 0.37	
	2	75	58.78 ± 32.69		75	5.99 ± 4.82		75	3.62 ± 1.77		75	2.88 ± 1.72		72	0.23 ± 0.20		75	0.35 ± 0.81	
	0 v.s. 1			0.004 (0.001)						0.65 (0.56)						0.24 (0.20)			0.10 (0.05)
	0 v.s. 2			0.007 (0.004)						0.024 (0.033)						0.008 (0.014)			0.030 (0.032)
	1 v.s. 2			0.90 (0.74)						0.008 (0.021)						0.17 (0.33)			0.39 (0.67)

N, allelic number for SNPs and subject number for gene dose; *P* value, permutation test *p* value for SNP and Kendall's coefficient of rank correlation for gene dose; SD, standard deviation. (P-value): p-value after removing 10 subjects who had had co-medications that influence the CYP2C19 enzymatic activity (permutation test for SNP and Kendall's coefficient of rank correlation for gene dose).

CYP2C19 is associated with methadone-induced adverse reactions in urine opiate test positive patients

In urine opiate test positive patients (Table 3), the rs4244285 allele type was significantly associated with QTc change in ECG (permutation $p = 0.024$), and with the total score of TESS (permutation $p = 0.021$). The G allele carriers of the extensive metabolizers had a greater QTc change, and a higher total score of TESS than the A allele carriers.

The *CYP2C19* GD was significantly associated with the QTc change interval in ECG ($p = 0.008$) and the current QTc ($p = 0.017$). However, the baseline QTc was not significantly associated with the *CYP2C19* GD (data not shown). The extensive metabolizers of (2 GD) had a greater QTc change than the intermediate (1 GD) or poor metabolizers (0 GD).

The *CYP2C19* GD was also significantly associated with the total score of TESS ($p = 0.018$) and QTc change in urine opiate test positive patients, but not in urine opiate test negative patients (Table 2). Among the eight adverse events commonly related to methadone, the *CYP2C19* GD was found to be significantly associated only with the fatigue score ($p = 0.007$) after adjusted with co-medication (Supplementary Table S1). The extensive metabolizers (2 GD) had a higher fatigue score than the poor metabolizers (0 GD) ($p = 0.025$).

Discussion

Considerable differences in the frequencies of the polymorphisms within the *CYP2C19* gene have been noted in different ethnic groups. For instance, the frequencies of the loss-of-function genotypes (e.g., in *2 and *3 in *CYP2C19*) are higher in Asians than Caucasians or Africans (Luo et al., 2006; Mizutani, 2003). In the current study cohort, the frequencies of *CYP2C19**2 and *3 were 31.5% and 4.2%, respectively, which were consistent with the report in a previous study in a Han Chinese population (Zhou et al., 2009). The allele frequencies of *CYP2C19*17* in Asians were approximately 0.5% (Tsai et al., 2010) to 0.64% (Wang et al., 2009), which were too low to be calculated in our ethnic group.

Genetic polymorphisms of *CYP2B6* have been reported to contribute to the methadone plasma level, but not to the treatment outcome (Crettol et al., 2005). In our previous studies, we also found that the *CYP2B6* genetic polymorphisms were associated with the *S*-methadone plasma level and its clearance (Wang et al., 2011). Polymorphisms in *CYP3A4* were associated with withdrawal symptoms and adverse reaction (Chen et al., 2011). In this study, functional SNPs composed of GD in *CYP2C19* showed associations with methadone dose, especially in urine opiate test negative patients. The intermediate and extensive metabolizers of *CYP2C19* required a higher methadone dose than the poor metabolizers. This result is supported by a few *in vitro* studies in the metabolism of methadone by CYP2C19 (Chang et al., 2011; Gerber et al., 2004; Totah et al., 2007). The interactions between *CYP2C19* and *CYP2B6* or *CYP3A4* did not show significant associations with methadone dose (data not shown), or its dose corrected plasma *R*-methadone or *R*-EDDP concentrations both in all patients and in urine opiate test negative patients (Supplementary Tables S4 and S5). As the dose of methadone may be determined by multiple genes (Fonseca et al., 2011; Hung et al., 2011), our current results indicate that the *CYP2C19* GD may play a role in the

TABLE 3. ASSOCIATION ANALYSES OF *CYP2C19* GENETIC VARIANTS AND QTC CHANGE, RATIOS OF PLASMA METHADONE OR METABOLITE/DOSE, AND SIDE EFFECT TOTAL SCORES IN URINE OPIATE TEST POSITIVE PATIENTS

SNP/Gene dose	*Allele/ Gene dose*	*QTc Change (ms)*			*Sum of Tess*			*R-Methadone/ dosage ratio*			*S-Methadone/ dosage ratio*			*R-EDDP/ dosage ratio*			*S-EDDP/ dosage ratio*		
		N	*Mean ± SD*	*p value*	*N*	*Mean ± SD*	*p value*	*N*	*Mean ± SD*	*P value*	*N*	*Mean ± SD*	*p-value*	*N*	*Mean ± SD*	*p-value*	*N*	*Mean ± SD*	*p-value*
rs4244285	A	55	8.96 ± 30.26	0.024 (0.021)	55	6.00 ± 6.67	0.021 (0.021)	55	3.54 ± 1.27	0.83 (0.83)	55	2.69 ± 1.26	0.96 (0.96)	52	0.25 ± 0.21	0.83 (0.83)	55	0.26 ± 0.16	0.09 (0.10)
	G	129	18.89 ± 25.51		129	8.87 ± 8.03		129	3.49 ± 1.77		129	2.68 ± 1.58		124	0.25 ± 0.24		127	0.37 ± 0.53	
rs4986893	A	8	16.00 ± 21.01	0.99 (1.00)	8	7.00 ± 4.00	0.72 (0.71)	8	3.70 ± 1.32	0.73 (0.73)	8	3.06 ± 1.35	0.48 (0.48)	7	0.39 ± 0.33	0.083 (0.082)	8	0.58 ± 0.59	0.10 (0.09)
	G	176	15.92 ± 27.62		176	8.06 ± 7.88		176	3.49 ± 1.64		176	2.66 ± 1.50		169	0.24 ± 0.23		174	0.33 ± 0.44	
Gene Dose	0	11	4.73 ± 25.11	0.008 (0.008)	11	5.00 ± 3.74	0.018 (0.018)	11	3.82 ± 1.48	0.12 (0.12)	11	2.88 ± 1.37	0.26 (0.26)	10	0.37 ± 0.24	0.73 (0.73)	11	0.29 ± 0.19	0.50 (0.50)
	1	41	12.61 ± 31.38		41	6.73 ± 7.42		41	3.43 ± 1.15		41	2.66 ± 1.24		39	0.22 ± 0.21		41	0.30 ± 0.31	
	2	40	22.40 ± 22.13		40	10.15 ± 8.47		40	3.50 ± 2.07		40	2.65 ± 1.77		39	0.25 ± 0.25		39	0.39 ± 0.61	
	0 v.s. 1			0.31 (0.31)			0.85 (0.85)												
	0 v.s. 2			0.020 (0.020)			0.08 (0.08)												
	1 v.s. 2			0.0499 (0.0499)			0.028 (0.028)												

N, allelic number for SNP s and subject number for gene dose; *P* value, permutation test *p* value for SNP and Kendall's coefficient of rank correlation for gene dose; (*P* value): *p* value after removing 10 subjects who had had co-medications that influence the CYP2C19 enzymatic activity (permutation test for SNP and Kendall's coefficient of rank correlation p-value for gene dose); SD, standard deviation.

methadone dosing strategy in the future clinical practice, particularly in Asians.

Clinically, methadone is usually administered in a racemic mixture with both *R*- and *S*-enantiomers. It has been suggested that the *R*-methadone is more efficacious in terms of the therapeutic effects and has less severe adverse events, such as QT prolongation, than *S*-methadone (Kristensen et al., 1996; Lin et al., 2009). Previous studies have demonstrated that *CYP2C19* preferentially metabolizes *R*-methadone (Gerber et al., 2004; Wang et al., 2010). In the present analyses, our results indicated that the *CYP2C19* GD was a sensitive indicator for methadone dose, and the dose-corrected plasma concentration of *R*-methadone and *R*-EDDP especially in urine opiate test negative patients. In addition, intermediate and extensive methadone metabolizers showed a lower dose-corrected plasma *R*-methadone concentration. This explains why the intermediate and extensive metabolizers required a higher methadone dose in order to maintain a therapeutic level of *R*-methadone in the plasma.

QTc prolongation is commonly found in patients undergoing methadone treatment, particularly in those receiving a high dosage of methadone (Fonseca et al., 2009). Although most early studies suggested that the methadone-induced QTc prolongation is dose dependent, the dosage range of methadone required to induce clinically significant QTc prolongation remains unclear (Ehret et al., 2007; Ehret et al., 2006; Kim and Manini, 2008; Piguet et al., 2004; Reddy et al., 2010). Other risk factors such as the concurrent use of CYP450 isoenzyme inhibitors and hepatic insufficiency, which result in an increase in the plasma concentration of methadone, have also been suggested to contribute to QTc prolongation (Piguet et al., 2004). Methadone, but not EDDP, may block the potassium voltage-gated channels, such as subfamily H (eag-related), member 2 (*HERG1* or *KCNH2*) channels, therefore it influences the QT interval (Katchman et al., 2002). A recent pharmacogenomic study reported for the first time that slow metabolizers, defined by *CYP2B6* genotypes, usually have a reduced ability to metabolize *S*-methadone, hence they show a higher association with QTc prolongation (Eap et al., 2007). These findings shed light on the possible contributions of genetic variants of the genes encoding the metabolic enzymes to the QTc interval prolongation in subjects under methadone treatment.

In association analyses of urine opiate test positive patients, we found significant associations between the genetic variations, the GD of *CYP2C19,* and the QTc change. The G allele of rs4244285 and the intermediate-extensive metabolizers, defined by the *CYP2C19* GD, are risk factors for a greater QTc change. No significant associations were found between the methadone doses (data not shown) nor the dose-corrected plasma concentrations of methadone and EDDP and QTc changes in urine opiate test positive patients. This result suggests that continued use of heroin, which led to a positive urine opiate test, might contribute to QTc change. As any significant change in QTc may lead to serious consequences including sudden death, we believe that the findings in this report are clinically important and should be noted for further studies for replication, especially in other populations.

In the adverse reaction association analyses, only fatigue was found to be associated with the GD of *CYP2C19* in urine opiate test positive patients. This indicated that continued use of heroin while under MMT may also contribute to adverse events.

We have considered the possible effects of co-occurrence of HIV infection in the MMT patients in this study because several HIV medications are known to inhibit the enzymatic activities of CYP2C19 and 3A4 (Burger et al., 2006; Hirt et al., 2008), thus would affect the metabolism of methadone (Ward et al., 2003). In fact, only three subjects in the present cohort had been treated with HIV medications. We have also considered the influence of other co-administrated medications on the activities of CYP2C19. Therefore, we removed the data from the 10 patients who were treated with such medications and re-analyzed the data. The results remain statistically significant. Furthermore, more than 95% of the subjects in the present study were tested positive for plasma hepatitis C viral infection antibody. However, the GD of *CYP2C19* remains significantly associated with QTc interval after the glutamic oxaloacetic transaminase (GOT) liver functional levels were adjusted in analysis (data not shown). In addition, noncompliance had been controlled in this study by only recruiting subjects who had been stabilized on methadone treatment and showed regular attendance at the clinic.

Conclusion

The genotype and allele variation of SNP rs4244285 in *CYP2C19* were significantly associated with the dose-corrected plasma concentrations of *R*-methadone in urine opiate test negative MMT patients. The allele type of *CYP2C19* SNP rs4244285 and GD were associated with the change in QTc interval measured by ECG and with the sum of TESS scores in urine opiate test positive patients. G allele carriers among the faster metabolizers may experience a larger QTc change and more severe side effects than the slower metabolizers who are A allele carriers in the allelic model. Using GD models, *CYP2C19* showed significant associations with methadone dose and the dose-corrected plasma concentrations of *R*-methadone and *R*-EDDP in urine opiate test negative patients. These results indicate that the functional genetic variations in the exon regions composed of GD in the *CYP2C19* gene may be useful indicators for the cardiac side effects of prolonged QTc intervals for the urine opiate test positive MMT patients. These findings also suggest that *CYP2C19* genetic polymorphisms may be indicators for methadone dose and plasma *R*-methadone and *R*-EDDP/dose ratios for urine opiate test negative MMT patients, and indicators for cardiac QTc change and side effects for urine opiate test positive patients. Further independent studies in other populations are warranted.

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Author Disclosure Statement

The authors declare that no competing financial interests exist.

References

Ansermot N., Albayrak O, Schlapfer J, et al. (2010). Substitution of (R,S)-methadone by (R)-methadone: Impact on QTc interval. Arch Intern Med 170, 529–536.

Barrett JC, Fry B, Maller J, and Daly MJ. (2005). Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 21, 263–265.

Bazett HC. (1920). An analysis of the time-relations of electrocardiograms. Heart 7, 353–370.

Blanken P, Hendriks VM, Koeter MW, van Ree JM, and van den Brink W. (2012). Craving and illicit heroin use among patients in heroin-assisted treatment. Drug Alcohol Depend 120, 74–80.

Burger D, Schwietert H, Colbers E, and Becker M. (2006). The effect of the CYP2C19* 2 heterozygote genotype on the pharmacokinetics of nelfinavir. Br J Clin Pharmacol 62, 250–252.

Byrne A, and Stimmel B. (2007). Methadone and QTc prolongation. Lancet 369, author reply 366–367.

Chang Y, Fang WB, Lin SN, and Moody DE. (2011). Stereoselective metabolism of methadone by human liver microsomes and cDNA-expressed cytochrome P450s: A reconciliation. Basic Clin Pharmacol Toxicol 108, 55–62.

Chen CH, Wang SC, Tsou HH, et al. (2011). Genetic polymorphisms in CYP3A4 are associated with withdrawal symptoms and adverse reactions in methadone maintenance patients. Pharmacogenomics 12, 1397–1406.

Crettol S, Deglon JJ, Besson J, et al. (2006). ABCB1 and cytochrome P450 genotypes and phenotypes: Influence on methadone plasma levels and response to treatment. Clin Pharmacol Ther 80, 668–681.

Crettol S, Deglon JJ, Besson J, et al. (2005). Methadone enantiomer plasma levels, CYP2B6, CYP2C19, and CYP2C9 genotypes, and response to treatment. Clin Pharmacol Ther 78, 593–604.

De Morais SM, Wilkinson GR, Blaisdell J, Meyer UA, Nakamura K, and Goldstein JA. (1994). Identification of a new genetic defect responsible for the polymorphism of (S)-mephenytoin metabolism in Japanese. Mol Pharmacol 46, 594–598.

Dickson EJ, and Stuart RC. (2003). Genetics of response to proton pump inhibitor therapy: Clinical implications. Am J Pharmacogenomics 3, 303–315.

Eap CB, Bourquin M, Martin J, et al. (2000). Plasma concentrations of the enantiomers of methadone and therapeutic response in methadone maintenance treatment. Drug Alcohol Depend 61, 47–54.

Eap CB, Crettol S, Rougier JS, et al. (2007). Stereoselective block of hERG channel by (S)-methadone and QT interval prolongation in CYP2B6 slow metabolizers. Clin Pharmacol Ther 81, 719–728.

Ehret GB, Desmeules JA, and Broers B. (2007). Methadone-associated long QT syndrome: Improving pharmacotherapy for dependence on illegal opioids and lessons learned for pharmacology. Expert Opin Drug Saf 6, 289–303.

Ehret GB, Voide C, Gex-Fabry M, et al. (2006). Drug-induced long QT syndrome in injection drug users receiving methadone: High frequency in hospitalized patients and risk factors. Arch Intern Med 166, 1280–1287.

Elkader AK, Brands B, Dunn E, Selby P, and Sproule BA. (2009). Major depressive disorder and patient satisfaction in relation to methadone pharmacokinetics and pharmacodynamics in stabilized methadone maintenance patients. J Clin Psychopharmacol 29, 77–81.

Ferguson RJ, De Morais SM, Benhamou S, B et al. (1998). A new genetic defect in human CYP2C19: Mutation of the initiation codon is responsible for poor metabolism of S-mephenytoin. J Pharmacol Exp Ther 284, 356–361.

Fonseca F, de la Torre R, Diaz L, et al. (2011). Contribution of cytochrome P450 and ABCB1 genetic variability on methadone pharmacokinetics, dose requirements, and response. PLoS ONE 6, e19527.

Fonseca F, Marti-Almor J, Pastor A, et al. (2009). Prevalence of long QTc interval in methadone maintenance patients. Drug Alcohol Depend 99, 327–332.

Gerbal-Chaloin S, Pascussi J, Pichard-Garcia L, et al. (2001). Induction of CYP2C genes in human hepatocytes in primary culture. Drug Metab Disposition 29, 242–251.

Gerber JG, Rhodes RJ, and Gal J. (2004). Stereoselective metabolism of methadone N-demethylation by cytochrome P4502B6 and 2C19. Chirality 16, 36–44.

Goldstein JA. (2001). Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. Br J Clin Pharmacol 52, 349–355.

Guy W. 1976. *ECDEU Assessment Manual for Psychopharmacology*, Revised ed. (U.S. Dept. of Health, Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, National Institute of Mental Health, Psychopharmacology Research Branch, Division of Extramural Research Programs, Rockville, MD).

Hirt D, Mentre F, Tran A, et al. (2008). Effect of CYP2C19 polymorphism on nelfinavir to M8 biotransformation in HIV patients. Br J Clin Pharmacol 65, 548–557.

Hung CC, Chiou MH, Huang BH, et al. (2011). Impact of genetic polymorphisms in ABCB1, CYP2B6, OPRM1, ANKK1 and DRD2 genes on methadone therapy in Han Chinese patients. Pharmacogenomics 12, 1525–1533.

Hutchinson MR, and Somogyi AA. (2004). (S)-(+)-methadone is more immunosuppressive than the potent analgesic (R)-(-)-methadone. Int Immunopharmacol 4, 1525–1530.

Ingelman-Sundberg M, Sim SC, Gomez A, and Rodriguez-Antona C. (2007). Influence of cytochrome P450 polymorphisms on drug therapies: Pharmacogenetic, pharmacoepigenetic and clinical aspects. Pharmacol Ther 116, 496–526.

Katchman AN, McGroary KA, Kilborn MJ, et al. (2002). Influence of opioid agonists on cardiac human ether-a-go-go-related gene K(+) currents. J Pharmacol Exp Ther 303, 688–694.

Kim HK, and Manini A. (2008). Methadone-associated sudden cardiac death? Am J Med 121, e11.

Kristensen K, Blemmer T, Angelo H, et al. (1996). Stereoselective pharmacokinetics of methadone in chronic pain patients. Ther Drug Monit 18, 221–227.

Leimanis E, Best BM, Atayee RS, and Pesce AJ. (2012). Evaluating the relationship of methadone concentrations and EDDP formation in chronic pain patients. J Anal Toxicol 36, 239–249.
Lin C, Somberg T, Molnar J, and Somberg J. (2009). The effects of chiral isolates of methadone on the cardiac potassium channel IKr. Cardiology 113, 59–65.
Luo HR, Poland RE, Lin KM, and Wan YJ. (2006). Genetic polymorphism of cytochrome P450 2C19 in Mexican Americans: A cross-ethnic comparative study. Clin Pharmacol Ther 80, 33–40.
Mizutani T. (2003). PM frequencies of major CYPs in Asians and Caucasians. Drug Metab Rev 35, 99–106.
NCBI 2011. Reference SNP(refSNP) Cluster Report.
NIH 2011. Therapeutic Drug Monitoring and Pharmacogenomics Study of Methadone Therapy (M0108).
Piguet V, Desmeules J, Ehret G, Stoller R, and Dayer P. (2004). QT interval prolongation in patients on methadone with concomitant drugs. J Clin Psychopharmacol 24, 446–448.
Reddy S, Hui D, El Osta B, et al. (2010). The effect of oral methadone on the QTc interval in advanced cancer patients: A prospective pilot study. J Palliat Med 13, 33–38.
Rodi C, Darnhofer-Patel B, Stanssens P, Zabeau M, and van den Boom D. (2002). A strategy for the rapid discovery of disease markers using the MassARRAY system. BioTechniques 32, S62–S69.
Tost J, and Gut IG. (2005). Genotyping single nucleotide polymorphisms by MALDI mass spectrometry in clinical applications. Clin Biochem 38, 335–350.
Totah RA, Allen KE, Sheffels P, Whittington D, and Kharasch ED. (2007). Enantiomeric metabolic interactions and stereoselective human methadone metabolism. J Pharmacol Exp Ther 321, 389–399.
Totah RA, Sheffels P, Roberts T, Whittington D, Thummel K, and Kharasch ED. (2008). Role of CYP2B6 in stereoselective human methadone metabolism. Anesthesiology 108, 363–374.
Tsai MH, Lin KM, Hsiao MC, et al. (2010). Genetic polymorphisms of cytochrome P450 enzymes influence metabolism of the antidepressant escitalopram and treatment response. Pharmacogenomics 11, 537–546.
Wang G, Lei HP, Li Z, et al. (2009). The CYP2C19 ultra-rapid metabolizer genotype influences the pharmacokinetics of voriconazole in healthy male volunteers. Eur J Clin Pharmacol 65, 281–285.
Wang JS, and DeVane CL. (2003). Involvement of CYP3A4, CYP2C8, and CYP2D6 in the metabolism of (R)- and (S)-methadone in vitro. Drug Metab Dispos 31, 742–747.
Wang PW, Wu HC, Yen CN, et al. (2012). Change in quality of life and its predictors in heroin users receiving methadone maintenance treatment in Taiwan: An 18-month follow-up study. Am J Drug Alcohol Abuse 38, 213–219.
Wang SC, Ho IK, Tsou HH, et al. (2011). CYP2B6 polymorphisms influence the plasma concentration and clearance of the methadone S-enantiomer. J Clin Psychopharmacol 31, 463–469.
Wang SC, Ho IK, Wu SL, et al. (2010). Development of a method to measure methadone enantiomers and its metabolites without enantiomer standard compounds for the plasma of methadone maintenance patients. Biomed Chromatogr 24, 782–788.
Ward BA, Gorski JC, Jones DR, Hall SD, Flockhart DA, and Desta Z. (2003). The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: Implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. J Pharmacol Exp Ther 306, 287–300.
Zhou Q, Yu XM, Lin HB, et al. (2009). Genetic polymorphism, linkage disequilibrium, haplotype structure and novel allele analysis of CYP2C19 and CYP2D6 in Han Chinese. Pharmacogenom J 9, 380–394.

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Abbreviations Used

Ap Cl = apparent clearance
CYP = cytochrome P-450
ECG = electrocardiogram
EDDP = *R*,*S*-2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
GD = gene dose
HPLC = high-performance liquid chromatography
MAF = minor allele frequency
MMT = methadone maintenance therapy
PPI = proton pump inhibitor
QTc = electrocardiogram of heart-rate-corrected QT
SAP = shrimp alkaline phosphatase
SNP = single nucleotide polymorphism
TESS = treatment emergent symptom scale