CYP2B6 Polymorphisms Influence the Plasma Concentration and Clearance of the Methadone *S*-Enantiomer

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Abstract: Methadone is a racemic compound composed of the *R*-form and *S*-form enantiomers. The drug is usually used in maintenance therapy for the heroin-addicted patients. In our previous study, we found that the cytochrome P-450 (CYP) isozyme 2B6 preferentially metabolizes the *S*-methadone enantiomer. We thus tested whether *CYP2B6* gene polymorphisms had any influence on the concentration or clearance of methadone. Ten single nucleotide polymorphisms within this gene region were evaluated in 366 patients undergoing methadone maintenance for at least 3 months. The plasma steady-state levels of racemic methadone and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine were then measured in these individuals. The rs10403955 (T allele in intron 1), rs3745274 (G allele in exon 4), rs2279345 (T allele in intron 5), and rs707265 (A allele in exon 9) *CYP2B6* allele types were found to be significantly associated with a higher clearance, a lower plasma concentration, and a lower concentration-to-dosage (*C*/*D*) ratio of (*S*)-methadone ($P < 0.0017$). Two haplotype blocks of a trinucleotide haplotype (rs8100458-rs10500282-rs10403955 in intron 1) and a hexanucleotide haplotype (rs2279342-rs3745274-rs2279343-rs2279345-rs1038376-rs707265 from intron 2 to exon 9) were constructed within *CYP2B6*. The major combinations of T-T-T and A-G-A-T-A-A of these particular haplotypes showed significant associations with the plasma concentrations of *S*-methadone and its *C*/*D* ratio ($P < 0.0001$, respectively). We conclude that genetic polymorphisms in the *CYP2B6* gene may therefore be indicators of the clearance, plasma concentration and *C*/*D* ratio of *S*-methadone.

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Methadone, a synthetic μ-opioid receptor agonist, is commonly used as a maintenance agent for opioid dependence and pain control.[1] In most countries, methadone is mainly administered as a racemic mixture of *R*- and *S*-enantiomers. The *R*-methadone is thought to contribute to most clinical effects because of its stronger activation of μ-opioid receptors.[2] On the other hand, *S*-methadone is reported to be associated with unwanted side effects. Recent studies have demonstrated that *S*-methadone is related to higher levels of dissatisfaction with methadone treatment among patients and is associated with the risk of QT interval prolongation.[3,4] These findings suggest that the enantiomers of methadone play important roles in determining treatment adherence and safety for subjects on methadone maintenance treatment (MMT).

Methadone is extensively metabolized in the liver through specific isoforms of the cytochrome P-450 enzyme system.[5] It has been reported that these isoforms include CYP2B6, CYP2C19, CYP3A4, and, to a lesser extent, CYP2D6.[5–7] The CYP isozymes also preferentially metabolize the 2 different methadone enantiomers, the *R*-form in the case of CYP2C19, whereas the *S*-form is preferably metabolized by CYP2B6.[5] CYP2B6 is a liver metabolic enzyme that contributes between 2% and 10% of the total hepatic metabolic content.[8] This enzyme is involved in the metabolism of the antineoplastic agents cyclophosphamide and ifosfamide,[9] the antiretroviral agent efavirenz,[10] and methadone.[11] It is noteworthy that genetic variations in the *CYP2B6* gene seem to have an association with drug responses and the adverse effects of these agents.[12] The *CYP2B6* gene maps to chromosome 19q13.2 and comprises 9 exons.[13] Genetic variants of *CYP2B6* have been analyzed previously in different ethnic groups[13] and also in relation to the biotransformation of different drugs.[14] In a study of a white ethnic group, it has been found that variant *6 of CYP2B6 may be an indicator for the metabolism of the *S*-enantiomer of methadone.[7]

In our previous study, we established a system for measuring the enantiomers of methadone and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP).[15] We found that there was significant interindividual variability in the steady-state plasma concentrations of the enantiomers of methadone and EDDP, and we speculated that genomic profiles might contribute to these variations and provide further insights into the mechanisms of methadone action. In our present study, we thus examined whether *CYP2B6* gene polymorphisms had any impact on the concentration and clearance of enantiomers of methadone and its metabolite in a Taiwanese MMT cohort. Moreover, we examined whether *CYP2B6* genetic variations are

associated with methadone treatment outcomes and adverse events in this sample MMT population.

MATERIALS AND METHODS

Subjects

This study was approved by the institutional review board of the National Health Research Institutes (Zhunan, Taiwan) and the 6 participating hospitals. These hospitals were treating around 2300 MMT subjects at the time of the study, which is 20% of the total MMT patient population in Taiwan. Written informed consent was obtained from all participants. The project has also been registered as a National Institutes of Health Clinical Trial (http://www.clinicaltrial.gov/ct/show/NCT01059747). A total of 366 heroin addicts on a once-daily MMT regimen were recruited during 2009. The inclusion criteria included an age of 18 years or above, undergoing MMT for at least 3 months with regular attendance for the past 7 days, and a methadone dosage adjustment of not more than 10 mg in the past 7 days. Comorbidities including physical and mental disorders requiring immediate treatment and pregnancy were exclusion criteria.

Assessments

Demographics, substance use histories and methadone treatment courses, including the dose and treatment duration, and the treatment compliance during the previous week were obtained from the available medical records. Information regarding current comedications was obtained either from the medical records or from the participants' reports. We used a Treatment Outcomes Profile, which is an interviewer-administered instrument, to assess the frequency of illicit substance and alcohol abuse during the previous 4 weeks.[16] The Treatment Emergent Symptoms Scale, an instrument used to assess the severity of 43 items of treatment-related adverse events, was also conducted by research nurses before the intake of methadone on each study day.[17]

Urine and Blood Tests

Urine specimens were sampled before daily methadone intakes. The morphine and amphetamine levels were measured in these samples via a fluorescence polarization immunoassay using an Integra 800 device (Roche Diagnostics, Basel, Switzerland). Blood samples of 12 mL were taken at around 24 ± 2 hours after the last methadone intake, the time at which the plasma concentration of methadone is likely to be at its lowest. These samples were then delivered to the Taipei Institute of Pathology (Taipei, Taiwan) for analysis of liver enzyme functions including glutamate oxaloacetate transaminase (reference range, <38 U/L), glutamic pyruvic transaminase (reference range, <41 U/L), and γ-glutamyl transpeptidase (reference range, 8–61 U/L). Serology profiles were also performed for human immunodeficiency virus antibodies, hepatitis C viral antibodies, the surface antigen of the hepatitis B virus, HBsAg, and HBsAg antibodies. Assays for methadone and its metabolites and DNA genotyping were undertaken at the National Health Research Institutes.

Analysis of Methadone and Its Metabolites in Plasma

The plasma concentrations of methadone and its metabolite EDDP enantiomers were measured using high-performance liquid chromatography using the settings described in our previous report.[18] The high-performance liquid chromatography system consisted of a Waters 2795 Alliance solvent pump (Milford, Mass) and autosampler, a Waters 2998 photodiode array detector, and an HP computer recorder installed with Waters Empower software. A Chiral-APG analytical column (5 μm, 100 × 3 mm; Chrom Tech, Cheshire, UK) was used with a CHIRAL-AGP guard column (10 × 3 mm; Chrom Tech). The column oven was set at 35°C. The mobile phase comprised 5% isopropanol, 95% sodium phosphate buffer (10 mM), and triethylamine (1 mL/L of mobile phase), with a final pH of 6.0. The flow rate was 0.5 mL/min.

Methadone, EDDP, and 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, Calif). After the conditioning of the column on a vacuum manifold (Waters), 800-μL aliquots of each serum 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 (0.01 g/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 chromatographed. The intraday and interday coefficients of variation were 3.3% and 6.6% for *R*-methadone, 2.5% and 5.6% for *S*-methadone, 1.6% and 3.9% for *R*-EDDP, and 2.8% and 5.5% for *S*-EDDP, respectively. The recovery rates for *R*-methadone, *S*-methadone, *R*-EDDP, and *S*-EDDP were 109.0% (7.6%), 96.7% (8.6%), 96.6% (6.6%), and 87.4% (3.2%), respectively. The recovery rate for the internal standard was 60.2% (4.8%).

CYP2B6 Single Nucleotide Polymorphism Selection and Genotyping

DNA from the patient subjects was extracted from whole blood lymphocyte pellets using a Puregene kit (Gentra Systems, Minneapolis, Minn). The selection of *CYP2B6* single nucleotide polymorphisms (SNPs) for analysis was based on 3 principle considerations: (i) literature reports[13] of polymorphisms in this gene that relate to heroin addiction in ethnic groups from Asia, (ii) tagSNPs within the *CYP2B6* gene on HapMap (http://www.hapmap.org/index.html.en) with minor allele frequencies above 0.1 and an r^2 cutoff at 0.8, and (iii) functional SNPs predicted by FastSNP.[19] All SNP genotypings were performed using matrix-assisted laser desorption/ionization-time of flight mass spectrometry.[20] Primers and probes flanking the SNPs were designed using SpectroDESIGNER software (Sequenom, San Diego, Calif). DNA fragments (100-300 bp) encompassing each SNP site were amplified by polymerase chain reaction (GeneAmp 9700 thermocycler; Applied Biosystems, Foster City, Calif) in accordance with the manufacturer's instructions.

After removal of the unincorporated dNTPs and inactivation of the shrimp alkaline phosphatase (SAP) from the polymerase chain reaction, primer extension was performed via the addition of the appropriate probe, Thermo Sequenase (Amersham Pharmacia, Piscataway, NJ), and a ddNTP/dNTP mixture. The reaction conditions comprised 55 cycles of denaturation at 94°C for 5 seconds, annealing at 52°C for 5 seconds, and extension at 72°C for 5 seconds. The various extension products were differentiated by matrix-assisted laser desorption/ionization-time of flight analysis. This genotyping method has been applied previously to a broad variety of clinical applications and shows sufficient sensitivity to score SNPs from small amounts of template.[21] The self-validation for each SNP showed 100% consistency in all genotyping plates. ABI TaqMan SNP genotyping assays (Applied Biosystems) were used for samples that required further validation.

Statistics

All statistical analyses were conducted using SAS software, Version 9.2 (SAS Institute, Inc, Cary, NC). The urine morphine

test and plasma methadone concentrations were analyzed using a nonparametric Mann-Whitney *U* test. Association analyses between genotype or allele type and plasma drug concentrations were calculated using a general linear model. For power analyses, the PROC GLMPOWER procedure was used. Correction for multiple testing was performed by calculation of adjusted *P* values corresponding to a nominal type 1 error of 5% using a permutation test with the MULTTEST procedure. The relationships between methadone and its metabolite enantiomers were assessed by Pearson correlation coefficients. The Hardy-Weinberg equilibrium test and haplotype (95% confidence intervals) association analyses were performed using HAPLOVIEW version 4.1.[22] The haplotypes and their frequencies were determined using PHASE 2.1.[23] The associations between haplotype and the serum drug concentrations were assessed using a linear regression model with PROC GENMOD. The global *P* value was calculated via a mixed model with the PROC MIXED procedure. $P < 0.05$ was considered statistically significant.

RESULTS

Demographics and Clinical Characteristics

The demographics and clinical characteristics for a total of 366 MMT subjects were analyzed. Males were predominant (n = 297, 81.1%) in this cohort. Mean age was 38 years, and almost all patients were smokers. The percentage of carriers of viral hepatitis C and B was 95% and 23%, respectively. Mean (SD) for the glutamate oxaloacetate transaminase, glutamic pyruvic transaminase, and γ-glutamyl transpeptidase were 52.1 (55.7), 61.1 (74.2), and 62.1 (102.1) U/L, respectively. Only 4.3% of participants showed hepatic function within reference limits. In the case of human immunodeficiency, 24% subjects were deemed to be susceptible to infection. Percentages of self-reported alcohol and illicit substance use in the previous weeks were 31.7% for alcohol, 63.9% for heroin, and 17.5% for methamphetamine (Supplemental Table 1, Supplemental Digital Content 1, Demographics and Clinical Characteristics of Methadone Maintenance, http://links.lww.com/JCP/A59).

The mean starting dose of methadone was 32.0 (11.2) mg/d (range, 15–120 mg/d), and the current maintenance dose was adjusted to 54.7 (28.1) mg/d (range, 5–160 mg/d) in the study cohort, with a mean treatment duration of 65 weeks. Ten patients had coadministered medications that could potentially interfere the pharmacokinetics of methadone (fluoxetine, n = 2; sertraline, n = 1; ritonavir, n = 2; atazanavir, n = 2; oxcarbazepine, n = 1; ciprofloxacine, n = 1; cimetidine, n = 1; and prednisolone, n = 1) (Supplemental Table 2, Supplemental Digital Content 2, List of Coeducations Among The Methadone Maintenance Patients Analyzed in this Study, http://links.lww.com/JCP/A60).[24,25]

Treatment Outcomes and Methadone Metabolism

Mean (SD) plasma concentrations of methadone and EDDP were measured at 193.1 (121.8) and 142.2 (99.1) ng/mL for *R*- and *S*-methadone, respectively, and 13.7 (15.1) and 14.6 (13.0) ng/mL for *R*- and *S*-EDDP, respectively. The relation coefficient (*r*) between *R*- and *S*-methadone was determined to be 0.82 (Pearson, $P < 0.0001$), and the correlation coefficient between the *R*- and *S*-EDDP to be 0.39 (Pearson, $P < 0.0001$).

An urine morphine test was used as a surrogate measurement of the methadone treatment outcomes in this study. Among our patient cohort samples, 50% were positive in the urine morphine test. The urine morphine-negative and-positive subjects had *R*-methadone concentrations of 205.9 (128.8) and 182.5 (113.7) ng/mL and *S*-methadone concentrations of 156.2 (113.0) and 129.9 (81.9) ng/mL, respectively. There were no significant differences found between these 2 groups (Mann-Whitney *U* test, $P = 0.093$ for *R*-methadone and $P = 0.10$ for *S*-methadone, respectively). After correcting for the methadone dosage, the concentration-to-dosage ratios (*C*/*D* ratio) of both *R*- and *S*-methadone were found to be significantly higher in the urine morphine test-negative group (4.1 [1.9] ng/mL per milligram vs 3.7 [2.7] ng/mL per milligram for *R*-methadone [Mann-Whitney *U* test, $P = 0.001$] and 3.0 [1.7] ng/mL per milligram vs 2.6 [1.5] ng/mL per milligram [Mann-Whitney *U* test, $P = 0.012$]).

The levels of methadone clearance were calculated from the apparent clearance (Ap Cl) determined using the following equation: Ap Cl (L/h per kilogram) = dose (mg) / steady-state plasma concentration (ng/mL) × 24-hour dosing interval/body weight (kg). Our results indicated that the clearance of both *R*- and *S*-methadone was associated with treatment outcomes. Subjects with a positive urine morphine test had a significantly higher methadone Ap Cl compared with patients who were negative in the urine morphine test (Mann-Whitney *U* test, $P = 0.0018$ for *R*-methadone; and Mann-Whitney *U* test, $P = 0.02$ for *S*-methadone).

Among our study cohort, constipation (67.8%) was the most prevailing adverse event of methadone, followed by sedation (47.0%) and a change in libido (30.3%). There was no significant association found between these common adverse events and the plasma concentration or *C*/*D* ratio of *R*- and *S*-methadone or EDDP methadone metabolism.

Single Locus of *CYP2B6* and Methadone Metabolism and Clearance

A total of 10 *CYP2B6* SNPs were genotyped in our 366 MMT patient cohort (Table 1). All of these polymorphisms were found to be in Hardy-Weinberg equilibrium. The SNPs rs10500282 (intron 1), rs10403955 (intron 1), rs3745274 (exon 4), rs2279343 (exon 5), rs2279345 (intron 5), rs1038376 (exon 9), and rs707265 (exon 9) showed a significant association with both the plasma *C*/*D* ratio and the concentrations of *S*-methadone (Table 2). The allele types showing a higher mean *C*/*D* ratio of *S*-methadone also demonstrated higher mean plasma *S*-methadone concentrations. However, only the rs2279345 SNP was found to be significantly associated with the *C*/*D* ratio of *R*-methadone in both genotype ($P = 0.02$, power = 0.71) and allelic type ($P = 0.0075$, power = 0.76).

The rs8100458 SNP showed a significant association with the *R*- and *S*-EDDP plasma levels for both the genotype ($P = 0.023$ and 0.037, respectively) and allelic types ($P = 0.01$ and 0.041, respectively).

With regard to methadone clearance, the *CYP2B6* SNPs rs10403955 (intron 1), rs3745274 (exon 4), rs2279345 (intron 5), rs1038376 (exon 9), and rs707265 (exon 9) showed a significant association with the Ap Cl of *S*-methadone (Table 3). The genotype and allelic type SNPs rs10403955 ($P = 0.037$ and 0.049, respectively), rs3745274 ($P = 0.03$ and 0.042, respectively), and rs2279345 ($P = 0.017$ and 0.016, respectively) showed a significant association with the Ap Cl of *R*-methadone. However, the power values were no more than 0.72 in these cases. The allelic types found to be associated with a higher mean Ap Cl for *S*-methadone also showed a higher mean Ap Cl for *R*-methadone.

Analysis of *CYP2B6* Haplotypes and Methadone Metabolism and Clearance

Three haplotypes were constructed from the *CYP2B6* SNPs using Haploview.[26] A trinucleotide block (rs8100458-rs10500282-rs10403955) at intron 1 (block 1) and a hexanucleotide block

TABLE 1. Validated Single Nucleotide Polymorphisms of the *CYP2B6* Gene Analyzed in This Study

SNP ID*	Chromosome Position†	Functional Allele	Role	Allele‡	MAF§	HWP‖	n¶
rs8100458	46192053	Intronic enhancer, tagSNP	Intron 1	T/C	0.44	0.72	366
rs10500282	46200282	tagSNP	Intron 1	T/C	0.19	0.63	366
rs10403955	46201278	tagSNP	Intron 1	T/G	0.18	0.42	365
rs2279342	46201967	*1D,*4C, intronic enhancer, tagSNP	Intron 2	A/T	0.14	1	366
rs3745274	46204681	G516T,*6,*7,*9,*13,*19,*20, missense, splicing regulation	Exon 4	G/T	0.18	0.71	366
rs2279343	46207103	A785G,*4,*6,*7,*13,*16,*19,*20, missense, splicing regulation	Exon 5	A/G	0.26	0.79	366
rs2279345	46207542	Intronic enhancer, tagSNP	Intron 5	C/T	0.31	1	365
rs1038376	46215849	tagSNP	Exon 9	A/T	0.14	0.79	366
rs707265	46215927	tagSNP	Exon 9	G/A	0.33	0.66	365
rs1042389	46215993	tagSNP	Exon 9	T/C	0.37	0.81	366

*According to the dbSNP database.

†Based on the National Center for Biotechnology Information Human Genome Build 36.

‡The allele under the slash is the minor allele.

§Minor allele frequency.

‖Hardy-Weinberg equilibrium test of the *P* value.

¶Subject number.

(rs2279342-rs3745274-rs2279343-rs2279345-rs1038376-rs707265) ranging from intron 2 to exon 9 (block 2) are shown in Supplemental Figure A (Supplemental Digital Content 3, http://links.lww.com/JCP/A61). The block 1 TTT haplotype combination was found to have the lowest plasma concentrations of *S*-methadone, the lowest *S*-methadone/methadone dose ratio, but the highest Ap Cl of this methadone enantiomer (Supplemental Table 3, Supplemental Digital Content 4, Regression Association Analyses of *CYP2B6* Haplotypes and *S*-methadone Metabolism, http://links.lww.com/JCP/A62). This is in contrast

TABLE 2. Association Analyses Between the Indicated *CYP2B6* SNPs and the Plasma *S*-Methadone Concentration/Methadone Dose Ratios

SNP ID	Genotype	N*	*S*-Met/Met Dose, ng/mL per Milligram	*P*	Power	Allele	n†	*S*-Met/Met Dose, ng/mL per Milligram	*P*	Power
rs10500282	CC	15	7.15 (3.86)	<0.0001	0.99	C	139	6.74 (3.42)	<0.0001	0.99
	TC	109	6.63 (3.32)			T	593	5.27 (3.00)		
	TT	242	4.97 (2.84)							
rs10403955	GG	15	7.13 (3.89)	<0.0001	0.99	G	134	6.84 (3.43)	<0.0001	>0.99
	GT	104	6.75 (3.32)			T	596	5.25 (2.99)		
	TT	246	4.93 (2.83)							
rs3745274	GG	247	4.92 (2.83)	<0.0001	>0.99	G	598	5.25 (3.00)	<0.0001	>0.99
	GT	104	6.81 (3.28)			T	134	6.88 (3.40)		
	TT	15	7.13 (3.89)							
rs2279343	AA	202	5.05 (2.73)	0.0029	0.87	A	542	5.32 (2.98)	0.0016	0.91
	AG	138	6.11 (3.53)			G	190	6.20 (3.46)		
	GG	26	6.44 (3.33)							
rs2279345	CC	172	6.55 (3.33)	<0.0001	>0.99	C	501	5.99 (3.27)	<0.0001	>0.99
	TC	157	4.77 (2.79)			T	229	4.59 (2.60)		
	TT	36	4.21 (2.11)							
rs1038376	AA	269	5.24 (3.01)	0.0022	0.88	A	629	5.39 (3.06)	0.0008	0.92
	AT	91	6.30 (3.23)			T	103	6.52 (3.42)		
	TT	6	8.21 (4.63)							
rs707265	AA	37	3.85 (2.05)	<0.0001	0.99	A	240	4.73 (2.72)	<0.0001	0.99
	GA	166	5.12 (2.90)			G	490	5.96 (3.25)		
	GG	162	6.39 (3.34)							

*Subject number.

†Allelic number.

P value indicates a nominal type 1 error of 5% using a permutation test; *S*-Met/Met dose, plasma *S*-methadone concentration/methadone dose ratios. Values in parenthesis represent standard deviation.

TABLE 3. Association Analyses Between *CYP2B6* SNPs and the Apparent Clearance of *S*-Methadone

SNP ID	Genotype	n*	Ap Cl *S*-Met,† L/h per Kilogram	*P*	Power	Allele	n‡	Ap Cl *S*-Met,† L/h per Kilogram	*P*	Power
rs10403955	GG	15	12.37 (8.58)	0.0047	0.84	G	134	13.07 (10.46)	0.0017	0.89
	GT	104	13.28 (11.00)			T	590	18.44 (18.62)		
	TT	243	19.55 (19.73)							
rs3745274	GG	244	19.63 (19.71)	0.0025	0.88	G	592	18.46 (18.61)	0.0011	0.92
	GT	104	12.98 (10.77)			T	134	12.84 (10.27)		
	TT	15	12.37 (8.58)							
rs2279345	CC	171	13.46 (10.34)	0.0002	0.96	C	497	15.73 (15.65)	<0.0001	0.97
	TC	155	20.74 (22.72)			T	227	21.17 (20.61)		
	TT	36	22.10 (15.33)							
rs1038376	AA	267	18.81 (19.18)	0.042	0.60	A	624	18.06 (18.29)	0.016	0.67
	AT	90	13.59 (10.92)			T	102	13.56 (10.84)		
	TT	6	13.29 (11.18)							
rs707265	AA	37	23.69 (14.77)	0.0008	0.93	A	238	20.86 (20.26)	0.0002	0.96
	GA	164	19.58 (22.26)			G	486	15.75 (15.77)		
	GG	161	13.81 (10.64)							

*Subject number.

†All values $\times 10^{-5}$.

‡Allelic number.

Ap Cl *S*-Met indicates apparent clearance of *S*-methadone; *P*, nominal type 1 error of 5% using a permutation test.

Values in parenthesis represent standard deviation.

to the block 1 TCG haplotype combination, which showed the lowest Ap Cl for both the *R*- and *S*-methadone enantiomers compared with all other combinations. The block 2 AGATAA haplotype combination had lower plasma concentrations of *S*-methadone and lower *S*-methadone/methadone dose ratios than the ATGCAG and ATGCTG combinations. A summary of these results is shown in Figure 1.

DISCUSSION

We genotyped 10 SNPs within the *CYP2B6* genetic region in our current study in an MMT patient cohort from Taiwan. The allele frequencies were comparable to those reported by HapMap among the Chinese population and were found to be in Hardy-Weinberg equilibrium (Table 1). These results suggest that the current sample size and the data obtained properly reflect a randomized sampling of the general Taiwanese population.

Most patients undergoing methadone treatment are administered racemic preparations that contain an equal amount of *S*- and *R*-enantiomers.[27] CYP2B6 has been reported to show a metabolic preference for *S*-methadone,[5] and we have also reported that *S*-methadone metabolizes faster when racemic methadone is incubated with CYP2B6.[18] It has been further reported in a study of whites that in the case of *CYP2B6*6* (G allele in rs2279343 and T allele in rs3745274), the **6* carriers have higher plasma concentrations of *S*-methadone than non-**6* carriers.[7] This finding is consistent with the results of our present study in which the **6* carriers in our MMT cohort showed higher plasma *S*-methadone concentration (Mann-Whitney *U* test, $P = 0.0016$) and *C*/*D* ratios for both *R*- and *S*-methadone (Mann-Whitney *U* test, $P = 0.0003$ and $P < 0.0001$, respectively) compared with the non-**6* carriers. Moreover, we further found in our present experiments that *CYP2B6 *6* is not the only variant that is significantly associated with the *S*-methadone concentration. The SNPs rs10500282 (intron 1), rs10403955 (intron 5), rs2279343 (exon 5; *4), rs2279345 (introns 5), rs1038376 (exon 9), and rs707265 (exon 9) also showed a significant association with the plasma *S*-methadone/methadone dose ratio. This indicates that, although the use of exon SNP markers in predicting metabolic enzymatic activity is essential, this approach has the drawback of low minor allele frequencies. Intronic SNPs should therefore be evaluated as potential indicators as they have higher minor allele frequencies. In addition, the *CYP2B6* gene harbors more than 1 SNP associated with plasma *S*-methadone when adjusted for dosage.

It has been reported previously both in vivo and in vitro that the expression of *CYP2B6* may be increased by alcohol.[28,29] This enzyme also shows increased expression in the brain regions containing cerebellar Purkinje cells and hippocampal pyramidal neurons in alcoholics and smokers.[29] In our current study, we compared the genotype and allelic types of the 10 selected *CYP2B6* SNPs between the alcohol and nonalcohol

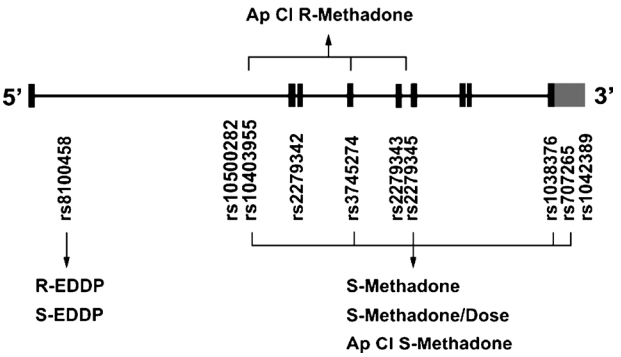

FIGURE 1. Graph summarizing the significant associations between the *CYP2B6* SNPs and metabolic phenotypes after methadone treatment.

users in our MMT patient cohort. However, none of these SNPs showed any association with the use of alcohol (data not shown).

The metabolic preference of CYP2B6 for *S*-methadone could also be observed in the genomic region of *CYP2B6* by genetic variant association analyses. Consistent findings from our single locus (Tables 2 and 3) and haplotype (Supplement Table 3, Supplemental Digital Content 4) association analyses further indicate a significant association between *CYP2B6* genetic variants or combinations of haplotypes and the *S*-methadone plasma concentration, the *S*-methadone concentration/methadone dose ratio, and the Ap Cl of *S*-methadone.

Among the CYP isozymes involved in methadone metabolism and clearance, CYP3A4 is considered to play the major role.[30,31] Hence, statements identifying methadone as a CYP3A4 substrate and warning about drug-drug interactions with the CYP3A4 inducer/inhibitors are included with the labeling of methadone products. Recent evidence suggests, however, that there is a more significant role of CYP2B6, but not CYP3A4, in human methadone clearance.[11] In our current study, we also found that the SNPs rs10403955, rs3745274, and rs2279345 are associated with the clearance of both *S*-methadone and *R*-methadone. In addition, the carriers of the *6 variation had a significantly higher plasma *C*/*D* ratio for both *S*- and *R*-methadone. The role of CYP2B6, not only in relation to stereoselective processing of *S*-methadone but also in overall methadone metabolism, should be taken into account when determining the clinical dose.

Coadministered medications that potentially interfere with methadone metabolism could have affected our current findings.[32–34] In our sample cohort, 10 (2.8%) MMT patients were coadministered with medications reported to induce or inhibit CYP3As, 2B6, 2C19, or 2D6. However, the overall results were unaffected by excluding this small subset of patients (data not shown).

Our current pharmacogenomic study has potentially limiting and confounding factors that are often associated with the analysis of a compound in a cross-sectional design. However, the potential confounding effects of noncompliance were mitigated by only recruiting subjects who had been stabilized on methadone treatment and showed regular attendance at therapy sessions. Furthermore, it has been previously hypothesized that the functional insufficiency of the hepatic microsomal system will interfere with the pharmacokinetics of methadone.[35] Indeed, more than 95% of our current subjects had chronic liver disease with impaired hepatic function, and the impact of this on the association between CYP2B6 and methadone metabolism warrants further investigation.

In summary, the results of our current experiments demonstrated that the *CYP2B6* SNPs rs10403955 (T allele), rs3745274 (G allele), rs2279345 (T allele), and rs707265 (A allele) are potential indicators of a faster clearance of the *S*-enantiomer of methadone. Furthermore, the SNPs rs10500282 (intron 1), rs10403955 (intron 1), rs3745274 (exon 4), rs2279343 (exon 5), rs2279345 (intron 5), rs1038376 (exon 9), and rs707265 (exon 9) are possible indicators of likely *S*-methadone/methadone dose ratios and plasma concentrations. Two *CYP2B6* haplotypes, block 1 (rs8100458-rs10500282-rs10403955) and block 2 (rs2279342-rs3745274-rs2279343-rs2279345-rs1038376-rs707265), were then constructed from the 10 SNPs under analysis. The block 1 TTT haplotype combination carriers had a higher Ap Cl but lower plasma concentrations of *S*-methadone and *S*-methadone/methadone dose ratios. In contrast, the block 2 AGATAA haplotype combination carriers had lower plasma concentrations of *S*-methadone and a lower *S*-methadone/methadone dose ratio. Our findings thus indicate that the *CYP2B6* SNPs strongly associate with the clearance and plasma concentrations of *S*-methadone but are not indicators of the major adverse effects of methadone such as constipation, sedation, or a reduced libido.

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AUTHOR DISCLOSURE INFORMATION

Chi-Shin Wu has lectured for and received honoraria from Eli-Lilly, Otsuka, and AstraZeneca. The other authors have no conflicts of interest to declare.

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