Title: Impact of genetic polymorphisms in *ABCB1*, *CYP2B6*, *OPRM1*, *ANKK1* and *DRD2* genes on methadone maintenance therapy optimization

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Abstract

The present study explored the integrative effect of genes encoding methadone pharmacokinetic and pharmacodynamic pathways on methadone maintenance doses. Genomic DNA was extracted from 321 opioid-dependent patients and 202 healthy controls, and realtime-PCR and PCR-RFLP were conducted to determine the genotypes. Pair-wise comparisons revealed that carriers of the variant *ABCB1* 3435C>T or *CYP2B6* 516G>T allele were more likely to require higher methadone dose than noncarriers (both $p<0.0001$). On the other hand, carriers of the variant *DRD2* -214A>G or 939C>T allele had a 2-fold chance of requiring lower methadone dose than noncarriers ($p=0.001$). Proportional odds regression with adjustment of cofactors demonstrated that *ABCB1*, *CYP2B6*, *OPRM1*, *ANKK1* and *DRD2* genetic variants were jointly correlated with optimal methadone dose (adjusted $r^2=53\%$). These findings provide a new insight that the interindividual variability of methadone dosage requirement is polygenetic and cannot be explained by single gene effect.

Keywords: methadone, polymorphism, *OPRM1*, *ANKK1*, *DRD2*

Introduction

Opiate dependency is a chronic, severe mental disorder; and methadone maintenance therapy is the standard treatment [1]. Optimal doses of methadone vary markedly among patients; and tailoring suitable dose for each individual is the key to safe and successful treatment [2]. The large interindividual variability of methadone maintenance dose may be partially explained by variations of multiple genes that are involved in the pharmacokinetic and pharmacodynamic pathways of methadone.

Methadone is a synthetic μ-opioid receptor agonist and is administered as a racemic mixture of (*R*)- and (*S*)-enantiomers. The (*R*)-methadone accounts for the major opioid effect [2]. After oral administration, methadone is rapidly absorbed, reaching maximum concentration at 2.5-4 hours, and its bioavailability ranges from 70% to 90 % [3]. Methadone is extensively metabolized in the liver by cytochrome P450 *CYP3A4*, *CYP2D6* and *CYP2B6* [4]. The elimination half-life of the racemic mixture ranges from 16 to 28 hours [5]. Previous studies have evaluated the effect of genetic variants in cytochrome P450 system on methadone metabolism and revealed that haplotype of *CYP2B6* 516G>T and 785A>G was correlated with higher methadone plasma trough concentration [4, 6].

Methadone is a substrate of P-glycoprotein, which is encoded by the *ABCB1* gene [7-9]. This is a highly polymorphic gene and more than 50 single nucleotide

polymorphisms (SNPs) have been identified [9]. P-glycoprotein is an efflux transporter expressed not only in tumor cells but also in the apical membranes of the intestine, the billiary canaliculi of the liver, the brush border of the renal proximal tubules, the luminal surface of blood capillaries of the brain (blood-brain barrier), and blood-tissue barriers [10]. Common variants in the *ABCB1* gene, such as 1236C>T, 2677G>T/A, and 3435C>T, have been shown to be associated with treatment responses and disease susceptibilities, though the results from different studies were controversial [11-16]. Carriers of the AGCTT haplotype (from positions 61, 1199, 1236, 2677, and 3435) in the *ABCB1* gene were demonstrated to be associated with lower methadone dosage requirement [17]. The methadone plasma concentration was also demonstrated to be associated with *ABCB1* 3435C>T [4]. In another study of heroin dependent Jewish patients, individuals with the TT-TT-TT genotype of 1236C>T, 2677G>T/A, and 3435C>T tended to use higher methadone dose [18]. However, the joint genetic effects of cytochrome P450 and the P-glycoprotein on methadone maintenance dose remain unclear.

As for the interindividual variability of the pharmacodynamics of methadone, polymorphisms in the gene coding for the μ-opioid receptor (*OPRM1*) have been considered as a primary contributor [19]. More than 20 variants with amino acid changes have been identified [20], and 118A>G has been associated with decreased

opioid effects, increased morphine dosage requirements, protection from opioid adverse effects, and susceptibility of drug addiction [21-24]. Moreover, in a study of the effect of (*R*)-methadone, the variant 118G allele was demonstrated to be associated with lower miotic potency in healthy subjects [25]. Thus, the decreased opioid potency caused by 118A>G may be also applied to methadone [23, 26].

Dopamine D_2 receptors (DRD2) have been considered as a key element of developing addictive behaviors, and the effect of variants in the *DRD2* gene on the DRD2 function have been investigated intensively [27-30]. Several studies have hypothesized that genetic variants altering DRD2 expression or function could be correlated to the required dosage and the response rate of methadone [29, 31]. Carriers of the C allele of *DRD2* 957C>T tended to have a higher nonresponse rate [29]; on the other hand, carriers of the T allele of 939C>T were more likely to use higher dosage of methadone [31]. Moreover, the variant T allele of the ankyrin repeat and kinase domain containing 1 (*ANKK1*) gene 2137C>T has been associated with poor treatment outcome of methadone maintenance therapy [32]; however, this association was not supported by other studies [29, 33].

To our knowledge, there has not yet study which explored integrative effects of methadone pharmacokinetics and pharmacodynamics related genes on methadone maintenance dosage. The current study simultaneously analyzed multiple relevant

genes to testify the possible synergistic effects of genetic variants on the dosage requirements of methadone.

Materials and Methods

Subjects

This study was approved by the institutional review board of China Medical University Hospital, a major medical center in Taiwan, and carried out in accordance with the Declaration of Helsinki.

Han Chinese patients with heroin dependence were recruited from the methadone clinic in China Medical University Hospital. Inclusion criteria included (1) having the capacity and willingness to give written informed consent; (2) being interviewed by an experienced research psychiatrist to confirm the diagnosis of heroin dependence by DSM-IV criteria [34]; (3) aging 20-60; (4) being within normal limits of EKG; and (5) receiving methadone for at least 6 months and keeping it dose unchanged for at least 4 weeks before enrollment. We also excluded patients receiving concurrent medications which may affect methadone metabolism. After complete description, 321 patients (253 men/68 women, 36.5±18.7 years old) were included after they gave written informed consent. For each patient the following clinical information was recorded: gender, weight (kg), height (cm), liver function, comorbidities and the daily dose of methadone.

Demographic characteristics of patients are shown in Table 1; they were divided into three groups based on their maximum stabilized methadone daily dose: less than

55 mg/day (low dose), between 55 and 99 mg/day (medium dose), and between 100 and 150 mg/day (high dose). The decision to split the methadone dose into three groups was according to the distribution of the dosage of included patients, and the basic characteristics among the three patient groups were not significantly different.

Random samples of 202 non-addict, Han Chinese health controls (105 men/97 women, aged 39.5±15.2 (mean ± SE)), without any major psychiatry and physical diagnosis, were enrolled for comparisons. They gave their consent to participate after procedures were explained to them. All were free of any Axis I or II psychiatric disorder, as determined by an experienced research psychiatrist according to DSM-IV [34]. All patients and controls were unrelated.

Genotyping

DNA was extracted from 3-10 ml of whole blood. Real-time PCR SNP analyses of *ABCB1* 2677G>T/A (rs2032582), *CYP2B6* 777C>T (rs45482602), *OPRM1* 118A>G (rs1799971) and 643+31G>A (rs9479757), *DRD2* 32+14266C>T (rs4648317), -214A>G (rs1799978), 811-83G>T (rs1076560) and 939T>C (rs6275) were carried out using the Applied Biosystem Assay on Demand reagents (Applied Biosystem, Foster City, Calif.) and were implemented using an ABI Prism 7900HT Sequence Detection System. On the other hand, the analyses of *ABCB1* 1236C>T (rs1128503) and 3435C>T (rs1045642), *CYP2B6* 516G>T (rs3745274), 785A>G

(rs2279343) and 1459C>T (rs3211371), *ANKK1* 2137C>T (rs1800497) and *GNB3* 825C>T (rs5443) were conducted using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as previously reported with minor modifications [4, 35-40].

Statistical analysis

To investigate the impact of the studied genetic variants on the maximum stabilized dosage of methadone, the prescribed dosages of patients were classified into three categories, less than 55 mg/day (low dose), between 55 and 99 mg/day (medium dose), and between 100 and 150 mg/day (high dose). Pairwise comparisons among the three dosage groups for the frequencies of alleles, genotypes, haplotypes and haplotype combinations were conducted using Pearson's chi-square test, Fisher's exact test and odds ratio (OR). In calculating OR, when a zero value appeared in the contingency table, a value of 0.5 was added to all cell counts of the table. Identification of haplotypes was performed using EM algorithm [41]. The standardized linkage disequilibrium values (D') and r^2 were calculated for measure of the linkage disequilibrium among these loci [42]. A p-value of less than 0.05 was considered to indicate statistical significance. Multiple comparisons were corrected using Bonferroni's method.

The proportional odds regression model was used to assess whether synergistic

effects existed between *ABCB1*, *CYP2B6*, *OPRM1*, *ANKK1* and *DRD2* genotypes with the adjustment for the liver function tests. The model selection procedures were undergone based on the Akaike Information Criterion (AIC) [43]. All data analyses were performed using SAS version 9.1.3 (SAS Inc, Cary, NC, USA).

Results

The allele and genotype frequencies of the *ABCB1*, *CYP2B6*, *ANKK1*, *GNB3*, *OPRM1* and *DRD2* polymorphic loci for patients and normal controls were listed in Table 2 and Table 3. The genotypic distributions were all consistent with Hardy-Weinberg equilibrium proportions. Significant linkage disequilibrium was detected among *ABCB1* 1236C>T, 2677G>T/A, and 3435C>T, between *CYP2B6* 516G>T and 785A>G, and among *ANKK1* 2137C>T, *DRD2* 32+1426C>T, -214A>G, 811-83C>A and 939C>T as indicated by high values of D' (>50; r^2>0.45; all p-values < 0.0001).

Association of genetic variants with patients or healthy controls

There was a significant trend toward patients carrying more frequently the minor genotype and allele of *DRD2* -214A>G (GG versus AA genotype: OR, 2.77; 95%CI: 1.10-6.97; p=0.030; G versus A allele: OR, 1.58; 95%CI: 1.14-2.21; p=0.007) (Table 2 and Table 3) or the CTACC and TCAAT haplotypes composed of *ANKK1* 2137C>T, *DRD2* 32+14266C>T, -214A>G, 811-83C>A, 939C>T (CTACC versus CCACC: OR, 14.61; 95%CI: 3.29-64.85; p=0.0004; TCAAT versus CCACC: OR, 38.58; 95%CI: 2.27-654.64; p=0.01) (Table 6).

Association of genetic variants with methadone maintenance therapy

Pairwise comparisons among the three dosage groups demonstrated that there

were significant association of maximum stabilized methadone dose with *ABCB1* 3435C>T, *CYP2B6* 516G>T, *DRD2* -214A>G and 939C>T (Table 2 and Table 3). Carriers of the variant *ABCB1* 3435C>T allele were at a 2.58-fold chance of requiring higher methadone dose than noncarriers (OR, 2.58; 95%CI: 1.66-3.99; p<0.0001) and the homozygous carriers conferred a 7.95-fold chance of requiring higher methadone dose (OR, 7.95; 95% CI: 2.96-21.33; p<0.0001). On the other hand, carriers of the variant *CYP2B6* 516G>T allele were at a 3-fold (1/0.31) chance of requiring lower methadone dose than noncarriers (OR, 0.31; 95%CI: 0.19-0.53; p<0.0001) and the homozygous carriers conferred a 7-fold (1/0.13) chance of requiring lower methadone dose (OR, 0.13; 95%CI: 0.04-0.42; p=0.0005). Similar results were observed in carriers of the variant *DRD2* -214A>G and 939C>T. Carriers of the variant *DRD2* -214A>G allele were at a 2-fold (1/0.4) chance of requiring lower methadone dose than noncarriers (OR, 0.40; 95%CI: 0.23-0.71; p=0.001) and the homozygous carriers were at a 14-fold (1/0.07) chance of requiring lower methadone dose (OR, 0.07; 95%CI: 0.009-0.56; p=0.01). Carriers of the variant *DRD2* 939C>T allele were at a 2-fold chance of requiring lower methadone dose than noncarriers (OR, 0.50; 95%CI: 0.32-0.76; p=0.001) and the homozygous carriers conferred a 3-fold chance of requiring lower methadone dose (OR, 0.27; 95%CI: 0.11-0.64; p=0.002).

Haplotypic analysis demonstrated that the haplotypes in *ABCB1*, *CYP2B6* and

ANKK1-DRD2 genes were associated with maximum stabilized methadone doses. Comparison of haplotype pattern distributions revealed that patients with CGT, TTC and TGT haplotypes composed of *ABCB1* 1236C>T, 2677G>T/A and 3435C>T were more likely to require higher methadone dose ($p<0.0001$; Table 4). For haplotypes in the *CYP2B6* gene, patients with TA and TG haplotypes composed of *CYP2B6* 516G>T and 785A>G were more likely to require lower methadone dose ($p<0.001$; Table 5). For haplotypes composed of *ANKK1* 2137C>T, *DRD2* 32+14266C>T, -214A>G, 811-83C>A and 939C>T, patients with CCACT, CTACT and TCAAC haplotypes were more likely to require lower methadone dose ($p<0.0001$; Table 6).

To further investigate the combined effect of pharmacokinetic and pharmacodynamic related genes on maximum stabilized methadone dose, proportional odds regression analysis was performed under adjustment of cofactors, such as liver function tests, height, weight and HIV infection. The most fitted model demonstrated that genetic variants in *ABCB1*, *CYP2B6*, *OPRM1*, *ANKK1* and *DRD2* genes and their interaction terms were significantly correlated with maximum stabilized methadone dose (adjusted r^2=53%; Table 7).

Discussion

The dosage optimization plays an important role in methadone maintenance therapy. Identifying genetic factors which may have impact on the dosage of methadone could help better individualized therapy. The present study suggests that multiple genes related to pharmacokinetic and pharmacodynamic pathways of methadone affect maintenance dose of methadone not only separately but also synergistically in patients with heroin addiction. In summary, the present study demonstrated that the *ABCB1* 3435C>T, *CYP2B6* 516G>T and *DRD2* -214A>G and 939C>T were significantly associated with methadone maintenance dose (all $p<0.001$). In addition, *ABCB1*, *CYP2B6, OPRM1* and *ANKK1-DRD2* genetic polymorphisms showed combined effects on methadone maintenance dose in the regression analysis and explained 53% variation of methadone maintenance dose. These results indicated that multiple genes were participated in the methadone maintenance dose requirements.

Previous studies have investigated the association of *ABCB1* gene with methadone plasma levels and methadone maintenance dose [4, 18]. Our findings further demonstrated that patients with CGT, TTC and TGT haplotypes composed of *ABCB1* 1236C>T, 2677G>T/A and 3435C>T tended to require higher methadone dose ($p<0.0001$). It has been suggested that the *ABCB1* polymorphism (3435C>T) is

not silent and can alter the stability and substrate specificity of P-glycoprotein [44, 45]. In addition, in patients with generalized epilepsy, the CSF concentrations of phenobarbital were significantly lower in subjects with CC genotype of 3435C>T [46]. These results were consistent with our findings of *ABCB1* gene and provided the possible mechanistic explanation. However, the effects of *ABCB1* genetic polymorphisms on treatment responses were controversial [17, 47-51]; and the inconsistent findings may have resulted from different definitions of treatment responses and influences of other genes than *ABCB1*. To compromise the defect of single gene analysis, the synergistic effect of pharmacokinetic (*CYP2B6*) and pharmacodynamic (*OPRM1, ANKK1, DRD2*) genes on the methadone maintenance dose was further investigated.

CYP2B6 is involved in the metabolism of numerous drugs, including bupropion, midazolam, ketamine, and methadone [38, 52-54]. It is expressed predominantly in the liver and is highly polymorphic [55]. The homozygous variant carriers of 785A>G exhibited a rapid metabolizer phenotype, whereas in the homozygous variant carriers of 785A>G in combination with 516G>T, a slow or poor metabolizer phenotype was observed [38, 56]. Anticipated low methadone dose requirements were observed in the present study. Patients with haplotype TG (composed of *CYP2B6* 516G>T and 785A>G) were at a 3-fold chance of requiring lower dose of methadone. The

significant association between methadone dose requirement and *CYP2B6* genetic variants provided further confirmation for *CYP2B6* was one of the important contributors to methadone metabolism.

The *ANKK1-DRD2* genetic polymorphisms have been identified to be associated with the methadone treatment outcomes and opiate addiction [29, 31]. The *DRD2* -214A>G was newly identified in the present study as a genetic variant modulating methadone dose requirement. This variant was demonstrated to be associated with nicotine abuse previously [30]. Another variant, 939C>T, was also associated with methadone dose requirement in the present study. Albeit inconsistent with previous study [31], our result may be supported from functional evaluation of the nearby variant, 957C>T, which alters RNA folding, decreases mRNA stability and protein synthesis, and reduces dopamine-induced upregulation of D2 receptor expression [57]. Due to the strong linkage with 957C>T, the functional consequence of 939C>T may be the same. The underlying molecular mechanism of this association requires further investigation.

The *OPRM1* 118A>G has been associated with opioid dependence [58, 59]; however, neither the association with opioid dependence nor association with methadone maintenance doses was detected in the present study and other studies in the single gene analysis [29, 60, 61]. Nonetheless, in the proportional odds regression

model, the *OPRM1* 118A>G, together with genetic variants in *ABCB1*, *CYP2B6*, *ANKK1* and *DRD2* genes, were demonstrated to be significantly associated with the maximum methadone maintenance doses. Therefore, in considering the genetic predictor of methadone maintenance doses, multiple genetic effects may be more biological plausible and explained more of the interindividual variances.

In conclusion, based on comprehensive analysis of pharmacokinetic and pharmacodynamic related genetic variants, the present study revealed that *ABCB1*, *CYP2B6, OPRM1* and *ANKK1-DRD2* genetic polymorphisms simultaneously modulated the maximum methadone maintenance doses and the haplotypes of *ANKK1-DRD2* genes were associated with the risk of opiate addiction. These results may provide further information regarding personalized pharmacotherapy approaches to methadone maintenance therapy.

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Table 1. Basic characteristics of patients on daily methadone maintenance therapy

	<55mg (n=92)	55-99 mg (n=150)	100-150 mg (n=79)
Gender (Male/Female)	71/21	122/28	60/19
Height (cm)	168.17±0.57	168.73±0.51	169.93±0.75
Weight (Kg)	64.67±1.04	65.08±0.80	64.68±0.93
Maximum Dose of Methadone (mg/day)	38.32±1.14	75.10±0.88	115.13±1.71
HIV-1 infection (yes/no)	0/92	5/150	2/79
sGOT	40.74±4.06	43.06±2.32	46.32±6.47
sGPT	47.72±4.54	55.55±4.17	56.84±8.12
rGT	46.48±6.43	37.48±2.24	41.72±7.16

All values, except for gender and HIV-1 infection, were expressed as mean ± standard error. These factors, including height, weight, sGOT, sGPT and rGT, were equally distributed among the three dosage groups.

Table 2. Genotype frequencies of *ABCB1*, *CYP2B6*, *ANKK1*, *GNB3*, *OPRM1* and *DRD2* polymorphic loci for patients under methadone maintenance therapy and healthy controls

genotypes	Low dose <55mg (n=92)		Medium dose 55-99mg (n=150)		High dose 100-150mg (n=79)		Healthy controls (n=202)	
	no.	%	no.	%	no.	%	no.	%
ABCB1								
1236C >T								
CC	7	7.61	24	16.00	16	20.25	27	13.37
CT	50	54.35	74	49.33	29	36.71	94	46.53
TT	35	38.04	52	34.67	34	43.04	81	40.10
2677G >A/T								
GG	22	23.91	32	21.33	23	29.11	48	23.76
GA	9	9.78	18	12.00	9	11.39	30	14.85
TA	8	8.70	20	13.33	6	7.59	26	12.87
TT	15	16.30	24	16.00	7	8.86	35	17.33
GT	36	39.13	55	36.67	33	41.77	55	27.23
AA	2	2.17	1	0.67	1	1.27	8	3.96
3435C> T[a]								
CC	30	32.61	54	36.00	9	11.39	34	16.83
CT	49	53.26	72	48.00	39	49.37	89	44.06
TT	13	14.13	24	16.00	31	39.24	79	39.11
CYP2B6								
516G>T[b]								
GG	44	47.83	98	65.33	57	72.15	88	43.56
GT	25	27.17	46	30.67	18	22.78	75	37.13
TT	23	25.00	6	4.00	4	5.06	39	19.31
785A>G								
AA	39	42.39	78	52.00	43	54.43	107	52.97
AG	38	41.30	63	42.00	31	39.24	76	37.62
GG	15	16.30	9	6.00	5	6.33	19	9.41
1459C>T								
CC	90	97.83	145	96.67	77	97.47	196	97.03
CT	2	2.17	5	3.33	2	2.53	6	2.97

TT	0	0.00	0	0.00	0	0.00	0	0.00
777C>A								
CC	84	91.30	139	92.67	77	97.47	197	97.52
CA	8	8.70	11	7.33	2	2.53	5	2.48
AA	0	0.00	0	0.00	0	0.00	0	0.00
ANKK1								
2137C>T								
CC	33	35.87	48	32.00	22	27.85	68	33.66
CT	41	44.57	72	48.00	36	45.57	108	53.47
TT	18	19.57	30	20.00	21	26.58	26	12.87
GNB3								
825C>T								
CC	23	25.00	41	27.33	27	34.18	40	19.80
CT	38	41.30	65	43.33	34	43.04	112	55.45
TT	31	33.70	44	29.33	18	22.78	50	24.75
OPRM1								
118A>G								
AA	49	53.26	71	47.33	33	41.77	89	44.06
AG	37	40.22	60	40.00	30	37.97	85	42.08
GG	6	6.52	19	12.67	16	20.25	28	13.86
643+31G>A								
GG	81	88.04	133	88.67	72	91.14	192	95.05
GA	11	11.96	17	11.33	7	8.86	10	4.95
AA	0	0.00	0	0.00	0	0.00	0	0.00
DRD2								
32+14266C>T								
CC	33	35.87	52	34.67	27	34.18	64	31.68
CT	41	44.57	69	46.00	38	48.10	98	48.51
TT	18	19.57	29	19.33	14	17.72	40	19.80
-214A>G[c, e]								
AA	56	60.87	89	59.33	60	75.95	148	73.27
AG	23	25.00	52	34.67	18	22.78	48	23.76
GG	13	14.13	9	6.00	1	1.27	6	2.97
811-83C>A								

CC	34	36.96	45	30.00	26	32.91	67	33.17
CA	38	41.30	72	48.00	38	48.10	103	50.99
AA	20	21.74	33	22.00	15	18.99	32	15.84
939C>T[d]								
CC	21	22.83	34	22.67	27	34.18	45	22.28
CT	34	36.96	81	54.00	39	49.37	100	49.50
TT	37	40.22	35	23.33	13	16.46	57	28.22

[a] Compared with patients taking low methadone dose, patients taking high methadone dose were more likely to have the TT genotype than the CC genotype (OR, 7.95; 95% CI: 2.96-21.33; p<0.0001). As well as compared with patients taking medium methadone dose, patients taking high methadone dose were more likely to have the TT genotype than CC genotype (OR, 7.75; 95%CI: 3.20-18.76; p<0.0001).

[b] Compared with patients taking low methadone dose, patients taking medium and high methadone dose were carried relatively less TT genotype (medium dose: OR, 0.12; 95% CI: 0.08-0.31; p<0.0001; high dose: OR, 0.13; 95% CI: 0.04-0.42; p=0.0005).

[c] Compared with patients taking low methadone dose, patients taking high methadone dose tended to carry relatively less GG genotype (OR, 0.07; 95%CI: 0.01-0.57; p=0.0125).

[d] Compared with patients taking low methadone dose, patients taking high methadone dose tended to carry less TT genotype than CC genotype (OR, 0.27; 95%CI: 0.12-0.64; p=0.0028).

[e] Patients carried more frequently the GG genotype (OR, 2.77; 95%CI: 1.10-6.97;

p=0.030).

Table 3. Allele frequencies of *ABCB1*, *CYP2B6*, *ANKK1*, *GNB3*, *OPRM1* and *DRD2* polymorphic loci for patients under methadone maintenance therapy and healthy controls

alleles	Low dose <55mg (n=92)		Medium dose 55-99mg (n=150)		High dose 100-150mg (n=79)		Healthy controls (n=202)	
	no.	%	no.	%	no.	%	no.	%
ABCB1								
1236C >T								
C	64	34.78	122	40.67	61	38.61	148	36.63
T	120	65.22	178	59.33	97	61.39	256	63.37
2677G> A/T								
G	89	48.37	137	45.67	88	55.70	181	44.80
T	74	40.22	123	41.00	53	33.54	151	37.38
A	21	11.41	40	13.33	17	10.76	72	17.82
3435C >T[a]								
C	109	59.24	180	60.00	57	36.08	157	38.86
T	75	40.76	120	40.00	101	63.92	247	61.14
CYP2B6								
516G>T[b]								
G	113	61.41	242	80.67	132	83.54	251	62.13
T	71	38.59	58	19.33	26	16.46	153	37.87
785A>G								
A	116	63.04	219	73.00	117	74.05	290	71.78
G	68	36.96	81	27.00	41	25.95	114	28.22
1459C>T								
C	182	98.91	295	98.33	156	98.73	398	98.51
T	2	1.09	5	1.67	2	1.27	6	1.49
777C>A								
C	176	95.65	289	96.33	156	98.73	399	98.76
A	8	4.35	11	3.67	2	1.27	5	1.24
ANKK1								
2137C>T								
C	107	58.15	168	56.00	80	50.63	244	60.40

T	77	41.85	132	44.00	78	49.37	160	39.60
GNB3								
825C>T								
C	84	45.65	147	49.00	88	55.70	192	47.52
T	100	54.35	153	51.00	70	44.30	212	52.48
OPRM1								
118A>G								
A	135	73.37	202	67.33	96	60.76	263	65.10
G	49	26.63	98	32.67	62	39.24	141	34.90
643+31G>A								
G	173	94.02	283	94.33	151	95.57	394	97.52
A	11	5.98	17	5.67	7	4.43	10	2.48
DRD2								
32+14266C>T								
C	107	58.15	173	57.67	92	58.23	226	55.94
T	77	41.85	127	42.33	66	41.77	178	44.06
-214A>G[c, e]								
A	135	73.37	230	76.67	138	87.34	344	85.15
G	49	26.63	70	23.33	20	12.66	60	14.85
811-83C>A								
C	106	57.61	162	54.00	90	56.96	237	58.66
A	78	42.39	138	46.00	68	43.04	167	41.34
939C>T[d]								
C	76	41.30	149	49.67	93	58.86	190	47.03
T	108	58.70	151	50.33	65	41.14	214	52.97

[a] Compared with patients taking low methadone dose, patients taking high methadone dose were more likely to carry the T allele than the C allele. (OR, 2.58; 95%CI: 1.66-3.99; p<0.0001). As well as compared with patients taking medium methadone dose, patients taking high methadone dose were more likely to carry the T allele than the C allele. (OR, 2.66; 95%CI: 1.78-3.96; p<0.0001).

[b] Compared with patients taking low methadone dose, patients taking high methadone dose tended to carry less T allele (OR, 0.31; 95%CI: 0.19-0.53; p<0.0001). Patients taking medium methadone dose were also tended to carry less T allele than patients taking low methadone dose (OR, 0.38; 95%CI: 0.25-0.58; p<0.0001).

[c] Compared with patients taking low methadone dose, patients taking high methadone dose tended to carry less G allele (OR, 0.40; 95%CI: 0.23-0.71; p=0.001).

[d] Compared with patients taking low methadone dose, patients taking high methadone dose tended to carry less T allele than C allele (OR, 0.49; 95%CI: 0.32-0.76; p=0.001).

[e] Patients carried more frequently the G allele (OR, 1.58; 95%CI: 1.14-2.21; p=0.007).

Table 4. Distribution of *ABCB1* haplotypes in three methadone dosage groups and healthy controls

haplotype (1236C >T -2677G >A/T-3435C>T)	Low dose <55mg (n=92)		Medium dose 55-99mg (n=150)		High dose 100-150mg (n=79)		Healthy controls (n=202)	
	no.	%	no.	%	no.	%	no.	%
CGC	40	21.74	60	20.00	2	1.27	6	1.49
CTC	5	2.72	10	3.33	6	3.80	1	0.25
CAC	9	4.89	24	8.00	0	0.00	4	0.99
CGT[a]	3	1.63	10	3.33	33	20.89	75	18.56
CTT	7	3.80	13	4.33	7	4.43	15	3.71
CAT	0	0.00	4	1.33	13	8.23	47	11.63
TGC	40	21.74	63	21.00	13	8.23	26	6.44
TTC[a]	7	3.80	16	5.33	33	20.89	108	26.73
TAC	9	4.89	6	2.00	3	1.90	12	2.97
TGT[a]	6	3.26	4	1.33	40	25.32	73	18.07
TTT	55	29.89	84	28.00	7	4.43	27	6.68
TAT	3	1.63	6	2.00	1	0.63	10	2.48

Ambiguous haplotypes were inferred via EM algorithm.

[a] Compared with patients taking low and medium methadone dose, patients taking high methadone dose were more likely to carry the CGT, TTC and TGT haplotypes (all $p<0.0001$).

Table 5. Distribution of *CYP2B6* haplotypes in three methadone dosage groups and healthy controls

haplotype (516G>T -785A>G)	Low dose <55mg (n=92)		Medium dose 55-99mg (n=150)		High dose 100-150mg (n=79)		Healthy controls (n=202)	
	no.	%	no.	%	no.	%	no.	%
GA	90	48.91	195	65.00	109	68.99	200	49.50
GG	23	12.50	47	15.67	23	14.56	51	12.62
TA[a]	26	14.13	24	8.00	8	5.06	90	22.28
TG[a]	45	24.46	34	11.33	18	11.39	63	15.59

Ambiguous haplotypes were inferred via EM algorithm.

[a] Compared with patients taking medium and high dose methadone, patients taking low methadone dose were more likely to carry the TA and TG haplotypes (all $p<0.001$).

Table 6. Distribution of *ANKK1-DRD2* haplotypes in three methadone dosage groups and healthy controls

haplotype (*ANKK1* 2137C>T-*DRD2* 32+14266C>T- -214A>G- 811-83C>A- 939C>T)	Low dose <55mg (n=92)		Medium dose 55-99mg (n=150)		High dose 100-150mg (n=79)		Healthy controls (n=202)	
	no.	%	no.	%	no.	%	no.	%
CCACC	4	2.17	16	5.33	26	16.46	28	6.93
CCACT[a]	28	15.22	42	14.00	6	3.80	59	14.60
CCGCC	1	0.54	0	0.00	5	3.16	0	0.00
CCGCT	12	6.52	20	6.67	0	0.00	16	3.96
CCGAT	3	1.63	10	3.33	0	0.00	5	1.24
CTACC[b]	2	1.09	7	2.33	39	24.68	2	0.50
CTACT[a]	46	25.00	59	19.67	1	0.63	119	29.46
CTGAT	5	2.72	6	2.00	0	0.00	0	0.00
TCACC	2	1.09	4	1.33	10	6.33	1	0.25
TCAAC[a]	30	16.30	48	16.00	0	0.00	73	18.07
TCAAT[b]	1	0.54	0	0.00	30	18.99	0	0.00
TCGAC	17	9.24	24	8.00	0	0.00	32	7.92
TCGAT	1	0.54	0	0.00	12	7.59	0	0.00
TTACT	3	1.63	6	2.00	1	0.63	0	0.00
TTAAC	15	8.15	40	13.33	10	6.33	37	9.16
TTAAT	0	0.00	0	0.00	15	9.49	1	0.25
TTGCT	4	2.17	0	0.00	0	0.00	0	0.00
Other 11 haplotypes[c]	10	5.43	18	6.00	3	1.90	31	7.67

Ambiguous haplotypes were inferred via EM algorithm.

[a] Compared with patients taking high dose methadone, patients taking medium and low methadone dose were more likely to carry the CCACT, CTACT and TCAAC haplotypes (all $p<0.0001$).

[b] Compared with healthy controls, patients were more likely to carry CTACC and TCAAT haplotypes (p=0.0004 and p=0.011, respectively).

[c] Rare haplotypes with frequencies were below 2% over four groups.

Table 7. Proportional odds regression analysis for factors related to methadone stabilized dosage

Regression parameters	Estimated values	standard errors	p-values
Intercept	-3.6001	1.0110	0.0004
ABCB1 1236C>T	-0.6932	0.3092	0.0249
ABCB1 3435C>T	-1.9804	0.4417	<0.0001
CYP2B6 516G>T	0.7019	0.3358	0.0366
OPRM1 118A>G	-0.4687	0.1890	0.0131
DRD2 811-83C>A	6.3570	1.1427	<0.0001
DRD2 939C>T	2.2945	0.5777	<0.0001
Interaction between *ABCB1* 1236C>T and 3435C>T	0.7743	0.2669	0.0037
Interaction between *ABCB1* 3435C>T and 2677G>A/T	0.2437	0.1228	0.0471
Interaction between *CYP2B6* 516G>T and 785A>G and 777C>A	4.5110	1.6860	0.0075
Interaction between *ANKK1* 2137C>T and *DRD2* 32+14266C>T	1.1355	0.4578	0.0131
Interaction between *ANKK1* 2137C>T and *DRD2* -214A>G	1.5797	0.6146	0.0102
Interaction between *ANKK1* 2137C>T and *DRD2* 811-83C>A	-2.0019	0.4400	<0.0001
Interaction between *ANKK1* 2137C>T and *DRD2* 939C>T	-0.8823	0.4084	0.0307

Interaction between *DRD2* 32+14266C>T and -214A>G	-4.1867	1.7018	0.0139
Interaction between *DRD2* 32+14266C>T and 811-83C>A	-1.9735	0.6314	0.0018
Interaction between *DRD2* -214A>G and 811-83C>A	-2.5156	0.8261	0.0023
Interaction between *DRD2* 811-83C>A and 939C>T	-3.5344	0.7141	<0.0001
Interaction between *DRD2* 32+14266C>T and -214A>G and 811-83C>A	2.8406	1.0905	0.0092
Interaction between *DRD2* 32+14266C>T and -214A>G and 939C>T	2.1229	1.0522	0.0436
Interaction between *DRD2* 32+14266C>T and 811-83C>A and 939C>T	1.0667	0.4395	0.0152
Interaction between *DRD2* -214A>G and 811-83C>A and 939C>T	1.4757	0.5250	0.0049
Interaction between *DRD2* 32+14266C>T and -214A>G and 811-83C>A and 939C>T	-1.4702	0.6446	0.0226