

ASSOCIATION OF *CYP2B6* AND *OPRM1* GENOTYPES WITH METHADONE DOSE REQUIREMENTS AND SERUM CONCENTRATIONS IN A VIETNAMESE COHORTS

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ABSTRACT

To characterize the prevalence of *CYP2B6* and *OPRM1* gene polymorphisms and evaluate their associations with serum methadone concentrations and dose requirements in Vietnamese patients undergoing methadone maintenance treatment (MMT). This cross-sectional study enrolled 200 patients with opioid dependence from multiple MMT clinics in Ninh Binh, Vietnam, between October 2023 and March 2024. Demographic, clinical, and behavioral data were collected using structured questionnaires. Blood samples were obtained for genotyping *CYP2B6* (rs2279343 [785A>G], rs3745274 [516G>T], rs8192709 [64C>T]) and *OPRM1* (A118G, rs1799971) using Sanger sequencing. Trough serum methadone concentrations were measured by validated liquid chromatography-tandem mass spectrometry (LC-MS/MS). Multivariable linear regression was performed to identify clinical and genetic factors associated with serum methadone concentrations, the concentration-to-dose ratio (CDR), and maintenance methadone dose. Among 200 participants (99% male, 96.9% Kinh ethnicity), the most common *CYP2B6* diplotypes were *1/*4 (33.3%) and *4/*6 (23.7%), with the *OPRM1* AG genotype present in 47.5%. Most patients (53.5%) had been on methadone treatment for over 3 years. In multivariable regression, the *1/*6 genotype was independently associated with lower maintenance dose ($\beta = -26.33$, 95% CI: $-51.97, -0.69$). The *2/*6 genotype was significantly associated with lower log serum methadone concentration ($\beta = -2.485$, 95% CI: $-3.024, -1.947$) and lower log CDR ($\beta = -2.595$, 95% CI: $-3.145, -2.046$). Most

demographic, behavioral, and *OPRM1* genotypes showed no significant associations. Specific *CYP2B6* genotypes and SNPs significantly influence methadone pharmacokinetics in Vietnamese patients receiving MMT, although actual daily dose remains the most important determinant of serum levels. These findings highlight the potential role of genotype-guided methadone dosing in optimizing therapy for opioid dependence in Vietnamese populations.

Keywords: *CYP2B6*, Methadone maintenance treatment, opioid use disorder, *OPRM1*, pharmacogenetics, serum methadone concentration

INTRODUCTION

The effectiveness and safety of methadone maintenance treatment (MMT) are challenged by considerable inter-individual variability in methadone pharmacokinetics and clinical response. Emerging evidence suggests that genetic factors significantly contribute to this variability. Among the genes studied, *OPRM1* and *CYP2B6* have been consistently identified as key determinants influencing methadone metabolism and treatment outcomes [1-3]. The *OPRM1* gene encodes the mu-opioid receptor, the primary target of methadone. A common functional polymorphism, A118G (rs1799971), has been associated with altered receptor binding and downstream signaling, potentially influencing both the efficacy and tolerability of opioid therapy [1,4,5]. In parallel, *CYP2B6* encodes the cytochrome P450 2B6 enzyme, which is responsible for the major metabolic pathway of methadone. The *CYP2B6* *6 allele, comprising rs3745274 and rs2279343, is linked to reduced enzymatic activity, slower methadone clearance, elevated plasma levels, and increased risk of toxicity [2,6-9].

The distribution of *CYP2B6* and *OPRM1* alleles varies widely across global populations, contributing to inter-individual differences in methadone metabolism and

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response. In East Asian countries such as China and Korea, the *CYP2B6* 4 allele is highly prevalent, often found in over 70% of individuals, while the 6 allele—associated with reduced enzyme activity and slower methadone metabolism—remains relatively uncommon [10,11]. In contrast, African and African-American populations exhibit much higher frequencies of the 6 allele, with some studies reporting prevalence rates up to 47% [6,12]. For *OPRM1*, the A118G polymorphism (rs1799971) shows significant ethnic variation as well. The G allele is most common in African populations, followed by Europeans, with reported frequencies ranging from 10% to over 20% in these groups [13,14]. However, few studies have investigated the prevalence or clinical implications of these polymorphisms specifically in methadone-maintained populations in Vietnam.

Given the lack of pharmacogenetic data in this context, a research gap exists in understanding how *OPRM1* and *CYP2B6* polymorphisms may affect methadone dosing and response among Vietnamese patients. The objective of this study is twofold: (1) to characterize the distribution of *OPRM1* and *CYP2B6* genotypes in a cohort of patients undergoing MMT in northern Vietnam; and (2) to assess their associations with key clinical outcomes, including serum methadone concentrations and maintenance dose requirements. Clarifying these relationships may support the development of genotype-informed strategies to optimize methadone therapy in Vietnam and similar resource-constrained settings.

MATERIALS AND METHOD

Study Design and Setting

This cross-sectional descriptive study was conducted from October 2023 to March 2024 at multiple sites in northern Vietnam, including methadone treatment facilities under the management of CDC Ninh Binh, Hanoi Medical University (Faculty of Medical Technology and the Center for Laboratory Quality Control), the Institute of Preventive Medicine, and the Department of Toxicology at the National Institute of Forensic Medicine. Ethical approval was obtained from the Institutional Review Board of Hanoi Medical University (Approval No. 980/GCN-HMUIRB, dated 18/10/2023).

Study sites were selected to ensure broad representation of methadone-maintained patients in Ninh Binh. Eligible participants were adults (≥ 18 years) diagnosed with opioid dependence according to Vietnamese guidelines and ICD10 criteria, and had been enrolled in methadone maintenance therapy (MMT) for at least 3 months. Exclusion criteria included ongoing use of other substances, current tuberculosis treatment, HIV infection under antiretroviral therapy (ART), and serious psychiatric disorders as assessed by the Kessler-6 scale.

To minimize variability in serum methadone concentrations due to missed or inconsistent dosing, all participants were recruited from clinics where methadone was administered under directly observed therapy (DOT) by trained healthcare staff. Patients were scheduled in advance for blood sampling to occur just prior to their next supervised methadone dose, aiming to approximate a 24-hour post-dose interval. The sampling procedure was strictly managed; however, due to the behavioral characteristics of the opioid-dependent population—who often face challenges with time adherence—minor deviations from the exact 24-hour interval were occasionally unavoidable. These deviations reflect real-world conditions and were considered during interpretation of the pharmacokinetic results. A total of 200 eligible and consenting patients were recruited for the study.

Data Collection and Measurement

Trained staff conducted structured interviews and reviewed medical records to collect demographic, clinical, and behavioral data. Variables included age, sex, education level, occupation, duration of methadone therapy, methadone dose history, smoking status, and alcohol use.

Venous blood was collected under aseptic conditions. For each participant, 3 mL of blood was drawn into an EDTA tube for DNA extraction and 3 mL into a serum tube for methadone quantification. Serum samples were stored at -80°C until analysis. Blood for methadone concentration measurement was collected close to the 24-hour post-dose time point, as described above.

Genomic DNA was extracted using the Exgene Blood SV kit (GeneAll Biotechnology). Four single nucleotide polymorphisms (SNPs) were analyzed: three in the *CYP2B6* gene—rs3745274, rs2279343, and rs8192709—and one in the *OPRM1* gene (rs1799971). Polymerase chain reaction (PCR) was performed using specific primers, followed by Sanger sequencing. Sequences were analyzed using ApE software and compared against NCBI reference sequences.

Serum methadone concentrations were quantified using validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) protocols, in accordance with U.S. FDA guidelines. Chromatographic separation was achieved using a UHPLC system (ExionLC) with a gradient of acetonitrile and formic acid over 6.1 minutes. Positive electrospray ionization mode was used for detection, with meperidine as the internal standard.

Variables and Outcomes

Demographic and behavioral variables included age, sex, education level, occupation, smoking status, and alcohol use. Clinical variables included methadone treatment

duration and daily dose. The primary outcome was serum methadone concentration (ng/mL), and the secondary outcome was the concentration-to-dose ratio (CDR), calculated as serum concentration (ng/mL) divided by daily dose (mg). Genetic variables were defined by alleles and diplotypes of *CYP2B6* and *OPRM1*.

While other pharmacogenetic markers such as *ABCB1* or *CYP3A4* may also influence methadone metabolism, this study focused on *CYP2B6* and *OPRM1* due to their established and direct roles in methadone pharmacokinetics. The exclusion of additional genes was a deliberate decision to maintain feasibility and ensure methodological focus for this pilot study.

Statistical Analysis

All data were analyzed using Stata version 17.0. Descriptive statistics summarized participant characteristics. Group comparisons were performed using chi-square tests for categorical variables and t-tests, ANOVA, or non-parametric equivalents for continuous variables. Hardy–Weinberg equilibrium (HWE) was assessed for each single nucleotide polymorphism (SNP) using chi-square tests to determine whether observed genotype frequencies deviated significantly from expected proportions. A p-value < 0.05 was considered evidence of deviation from HWE. To assess associations between genetic and clinical variables with methadone dose, serum methadone concentration (log-transformed), and concentration-to-dose ratio (CDR), multivariable linear regression models were constructed. Model fit was evaluated using R² values. Statistical significance was set at a two-sided p-value < 0.05.

RESULTS

Among the 200 participants, the vast majority were male (99.0%) and aged between 35 and 54 years. Nearly half had completed secondary school (47.5%), most were married (66.2%) and had children (70.1%). The most common occupation reported was housework or informal labor (55.2%). With respect to methadone treatment duration, over half (53.5%) had been on therapy for more than 3 years, while 26.3% had received treatment for less than 1

year, and 20.2% for 1 to 3 years. Longer treatment duration was significantly associated with older age ($p = 0.029$), Kinh ethnicity ($p = 0.013$), having children ($p = 0.039$), and higher mean age ($p = 0.011$).

Table 1 shows that participants with shorter treatment durations (<3 years) received higher initial methadone doses (approximately 21 mg/day), compared to those treated for over 3 years (18.8 mg/day; $p = 0.021$). However, maintenance doses and serum methadone concentrations were similar across groups, with mean serum levels ranging from 235.0 to 285.1 ng/mL ($p = 0.124$). The concentration-to-dose ratio (CDR), calculated as serum concentration (ng/mL) divided by maintenance dose (mg/day), had a mean value of 6.6 ± 3.7 ng/mL/mg and did not significantly differ across treatment duration groups ($p = 0.801$), indicating relatively consistent methadone pharmacokinetics across patients.

Table 2 presents the distribution of *CYP2B6* and *OPRM1* genotypes. The most prevalent *CYP2B6* diplotypes were *1/*4 (33.3%) and *4/*6 (23.7%), with allele *4 observed in 81.8% of the sample. At the single nucleotide polymorphism (SNP) level, the most frequent genotypes were GG at rs3745274 (52.0%), AG at rs1799971 (47.5%), and CC at rs8192709 (88.4%). Hardy–Weinberg equilibrium (HWE) tests revealed that while most loci conformed to expected distributions, rs3745274 and rs2279343 deviated from equilibrium, a pattern consistent with findings in Iranian methadone-maintained populations reported by Sara Sadat Aghabozorg Afjeh and colleagues [15].

In the multivariable regression models, several significant associations were observed. The *CYP2B6* *1/*6 genotype was associated with a significantly lower maintenance methadone dose (coefficient = -26.33 ; 95% confidence interval: -51.97 to -0.69). In addition, the *2/*6 genotype was significantly associated with lower log-transformed serum methadone concentrations (coefficient = -2.485 ; 95% CI: -3.024 to -1.947) and lower log CDR (coefficient = -2.595 ; 95% CI: -3.145 to -2.046). Higher final methadone dose also predicted increased serum methadone concentrations (coefficient = 0.019 ; 95% CI: 0.015 to 0.022). Other variables, including age, treatment duration, and *OPRM1* genotype, were not sig-

Table 1. Clinical, Behavioral, and Methadone Concentration Characteristics.

Variable	Categories	Duration of treatment				p-value
		<1 year	1–3 years	>3 years	Total	
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
Initial methadone dose (mg/day)		21.0 (8.6)	21.3 (8.6)	18.8 (4.7)	19.9 (6.8)	0.021
Maintenance methadone dose (mg/day)		58.6 (27.2)	51.0 (28.1)	51.2 (29.1)	53.3 (28.4)	0.182
Serum methadone (ng/mL)		285.1 (207.0)	275.3 (230.7)	235.0 (208.0)	257.6 (212.6)	0.124
CDR		5.0 (3.2)	5.4 (3.9)	4.8 (3.2)	6.6 (3.7)	0.801

Table 2. Distribution of Genotype, Allele, and SNP by Duration of Methadone Treatment.

Variable	Categories	Duration of treatment				p-value
		<1 year	1–3 years	>3 years	Total	
		N (%)	N (%)	N (%)	N (%)	
CYP2B6 genotype	*1/*1	2 (3.9)	1 (2.5)	2 (1.9)	5 (2.5)	0.762
	*1/*2	4 (7.7)	1 (2.5)	4 (3.8)	9 (4.5)	0.424
	*1/*4	14 (26.9)	15 (37.5)	37 (34.9)	66 (33.3)	0.499
	*1/*6	5 (9.6)	4 (10.0)	10 (9.4)	19 (9.6)	0.995
	*2/*4	3 (5.8)	2 (5.0)	8 (7.5)	13 (6.6)	0.827
	*2/*6	0 (0.0)	0 (0.0)	1 (0.9)	1 (0.5)	0.647
	*4/*4	5 (9.6)	5 (12.5)	7 (6.6)	17 (8.6)	0.501
	*4/*6	17 (32.7)	9 (22.5)	21 (19.8)	47 (23.7)	0.198
	*4/*9	5 (9.6)	4 (10.0)	10 (9.4)	19 (9.6)	0.995
	*6/*6	5 (9.6)	3 (7.5)	15 (14.2)	23 (11.6)	0.466
	*6/*9	0 (0.0)	1 (2.5)	4 (3.8)	5 (2.5)	0.365
Allele presence	Allele *1	25 (48.1)	21 (52.5)	53 (50.0)	99 (50.0)	0.915
	Allele *2	7 (13.5)	3 (7.5)	13 (12.3)	23 (11.6)	0.645
	Allele *4	44 (84.6)	35 (87.5)	83 (78.3)	162 (81.8)	0.364
	Allele *6	27 (51.9)	17 (42.5)	51 (48.1)	95 (48.0)	0.668
	Allele *9	5 (9.6)	5 (12.5)	14 (13.2)	24 (12.1)	0.807
rs3745274 [516G>T]	GG	25 (48.1)	23 (57.5)	55 (51.9)	103 (52.0)	0.375
	TT	5 (9.6)	4 (10.0)	19 (17.9)	28 (14.1)	
	GT	22 (42.3)	13 (32.5)	32 (30.2)	67 (33.8)	
rs2279343 [785A>G]	AA	3 (5.8)	1 (2.5)	3 (2.8)	7 (3.5)	0.597
	AG	22 (42.3)	21 (52.5)	58 (54.7)	101 (51.0)	
	GG	27 (51.9)	18 (45.0)	45 (42.5)	90 (45.5)	
rs8192709 [64C>T]	CC	45 (86.5)	37 (92.5)	93 (87.7)	175 (88.4)	0.645
	CT	7 (13.5)	3 (7.5)	13 (12.3)	23 (11.6)	
OPRM1	AA	21 (40.4)	9 (22.5)	38 (35.8)	68 (34.3)	0.475
	AG	23 (44.2)	22 (55.0)	49 (46.2)	94 (47.5)	
	GG	8 (15.4)	9 (22.5)	19 (17.9)	36 (18.2)	

nificantly associated with methadone-related outcomes. The explanatory power of the regression models was modest, with R^2 values of 0.1062 for methadone dose, 0.4435 for serum methadone concentration, and 0.1114 for CDR (Table 3).

DISCUSSION

This study found that specific *CYP2B6* genotypes, particularly *1/*2 and *1/*6, were associated with differences in methadone dose and serum concentration among patients on methadone maintenance. Most other genetic and clinical factors showed no significant association with methadone pharmacokinetics. These results suggest that certain genetic variants may be useful for individualizing

methadone therapy, though much of the variability remains unexplained and warrants further research.

In terms of genotype distribution, the most frequent *CYP2B6* diplotype in our Vietnamese cohort was *1/*4, accounting for approximately one-third of participants, followed by *4/*6. Less common genotypes included *1/*1, *1/*2, and *6/*9, each representing less than 10% of the sample. The predominance of the *1/*4 and *4/*6 diplotypes, as well as the high prevalence of the *4 allele (present in over 80% of participants), is broadly consistent with other studies conducted in East Asian populations, such as those in China and Korea^{10,11}. In contrast, the *6 allele—which is associated with slower methadone metabolism and higher plasma concentrations—is much more prevalent in African and African-American popula-

Table 3. Multivariable Regression Analysis of Factors Associated with Methadone Dose, Serum Methadone Concentration, and Concentration-to-Dose Ratio (CDR).

Factors	Maintenance methadone dose		Log (Serum methadone concentration)		Log (CDR)	
	Coef.	95%CI	Coef.	95%CI	Coef.	95%CI
Age (per year)	-0.15	-0.65, 0.36	0.003	-0.009, 0.014	0.008	-0.003, 0.019
Duration of treatment (vs < 1 year^a)						
1–3 years	-7.29	-19.21, 4.62	-0.012	-0.313, 0.289	-0.005	-0.305, 0.294
>3 years	-4.25	-14.12, 5.62	-0.120	-0.334, 0.094	-0.096	-0.311, 0.119
Initial dose (per mg/day)	0.45	-0.04, 0.95	-0.001	-0.023, 0.021	-0.005	-0.027, 0.016
Maintenance dose (mg/day)	–	–	0.019*	0.015, 0.022	-0.001	-0.004, 0.002
Genotype (Yes vs No^a)						
*1/*1	-18.13	-60.07, 23.81	-0.127	-0.796, 0.542	0.054	-0.682, 0.791
*1/*2	12.78	-8.33, 33.88	0.205	-0.191, 0.602	0.165	-0.211, 0.541
*1/*4	-18.05	-43.21, 7.11	-0.273	-0.803, 0.256	-0.285	-0.830, 0.259
*1/*6	-26.33*	-51.97, -0.69	-0.132	-0.720, 0.455	-0.135	-0.722, 0.452
*2/*4	-26.67	-54.75, 1.40	-0.604	-1.347, 0.139	-0.373	-1.038, 0.291
*2/*6	2.59	-23.88, 29.05	-2.485*	-3.024, -1.947	-2.595*	-3.145, -2.046
*4/*4	0.23	-34.14, 34.60	-0.286	-0.849, 0.278	-0.230	-0.797, 0.337
*4/*6	-10.92	-34.16, 12.31	-0.162	-0.595, 0.271	-0.159	-0.603, 0.285
*6/*6	-16.58	-42.71, 9.56	-0.146	-0.663, 0.371	-0.106	-0.640, 0.429
*6/*9	-9.86	-41.05, 21.32	-0.076	-0.773, 0.620	-0.088	-0.738, 0.562
OPRM1 (vs AA^a)						
AG	4.04	-4.68, 12.75	-0.017	-0.250, 0.215	0.003	-0.224, 0.231
GG	2.81	-9.32, 14.93	0.074	-0.217, 0.366	0.086	-0.188, 0.361
R ²	0.1062		0.4435		0.1114	

* $p < 0.05$; ^a reference group

tions, with frequencies reaching up to 47% in some cohorts, compared to the lower frequencies observed in both our Vietnamese sample and other Asian studies [6,12]. These ethnic differences in *CYP2B6* allele and diplotype distribution may contribute to the variability in methadone pharmacokinetics and dosing requirements reported across global populations. For the *OPRM1* gene, the AG genotype at rs1799971 (A118G) was most common in our cohort, with AA and GG genotypes being less prevalent. This distribution is consistent with published data from other Asian populations but differs from frequencies reported in European and especially African groups, where the G allele is more common [13,14]. The *OPRM1* A118G polymorphism has clinical significance, as the G allele has been associated with reduced receptor function, higher methadone plasma levels, and increased risk of poor treatment response or toxicity in several studies [14,16]. Notably, while the prevalence of the A118G allele in European populations is typically 10–15%, it is higher in African populations and generally lower in East Asian groups—findings which align with our data and those

of previous research [12,13]. Overall, the distribution of *CYP2B6* and *OPRM1* genotypes in our Vietnamese sample reflects expected patterns for East Asian populations but differs substantially from those observed in African and Western populations. This underscores the importance of considering ethnic and population-specific pharmacogenetic differences in the management of methadone maintenance therapy. As highlighted by recent studies, such as Twesigomwe et al. (2023) [12] and Chen et al. (2022) [11], the combination of *CYP2B6* and *OPRM1* genotype distributions, as well as additional genetic variants (e.g., ABCB1), may influence both methadone plasma concentrations and treatment outcomes, reinforcing the value of region-specific research and genotype-guided approaches to opioid dependence treatment.

Our study found that, among clinical and genetic factors, the *CYP2B6**1/*6 genotype was significantly associated with a lower final methadone dose, consistent with previous research. Polymorphisms in the *CYP2B6* gene, particularly *1/*6 and *2/*6, have been widely recognized as major determinants of methadone pharmacokinetic

variability. Literature has demonstrated that individuals carrying these variants display reduced enzyme activity, resulting in decreased metabolic clearance of methadone and consequently higher plasma drug concentrations at standard doses [17]. Surprisingly, our data showed that carriers of the *CYP2B6**2/*6 genotype had significantly lower log serum methadone concentrations and lower log CDR, which is contrary to much of the published literature. Typically, *2/*6 is associated with reduced methadone metabolism, leading to higher serum concentrations and an increased CDR [10,15,18]. Most studies report that these alleles, by reducing enzyme activity, slow methadone clearance and result in higher steady-state levels and a higher CDR, particularly for the S-enantiomer of methadone [3,7,19]. The presence of the 2 allele, on the other hand, has been less extensively studied and its effect on methadone pharmacokinetics is not well defined. The unexpected finding of reduced serum concentrations in *2/*6 carriers in our study could reflect unique genetic backgrounds or environmental influences in the Vietnamese population. Potential explanations include unmeasured gene–gene or gene–environment interactions, dietary or co-medication factors influencing enzyme expression (phenoconversion), or subtle differences in treatment practices such as formulation, absorption, or adherence variability. Given the inconsistencies with previously published pharmacogenetic data, our findings underscore the importance of conducting population-specific studies and caution against the universal application of pharmacogenetic interpretations across diverse ethnic groups. Future studies incorporating enantiomer-specific methadone measurement (e.g., LC-MS/MS) and broader genotype panels (e.g., *ABCB1*, *CYP3A4*) are needed to clarify these complex interactions and better guide genotype-informed methadone therapy.

The *OPRM1* A118G polymorphism has been extensively studied for its role in modulating opioid response, particularly in pain management and addiction. While G allele carriers often require higher doses of opioids and report fewer side effects such as nausea [20], the specific impact of this variant on methadone pharmacokinetics remains unclear. Several studies have suggested that methadone, due to its unique dual mechanism of action (as both a μ -opioid receptor agonist and NMDA receptor antagonist), may be less influenced by the A118G variant compared to other opioids [21]. A genome-wide association study in European-Americans found no significant association between A118G and methadone dose requirements, while other loci were implicated in African-American populations [22]. Additionally, recent research indicates that methadone may be particularly suitable for G allele carriers precisely because its efficacy is relatively unaffected

by this polymorphism [21]. Although the A118G variant has been associated with altered brain connectivity and opioid-related behaviors, such neurobiological effects do not appear to directly influence serum methadone concentrations [23,24]. Taken together, these findings support our observation of no significant association between *OPRM1* A118G and methadone serum levels, reinforcing the view that this polymorphism, while important in opioid response broadly, may have limited pharmacokinetic impact in the context of methadone maintenance treatment.

The implications of these findings are twofold. First, they reinforce the need for a precision medicine approach in MMT, particularly in ethnically distinct populations such as Vietnam, where *CYP2B6* allele frequencies may differ significantly from those assumed in Western pharmacogenetic guidelines. Second, although our results suggest that genetic testing could help identify individuals with atypical dose requirements or suspected poor metabolizer status, the overall incremental explanatory power of genetic variables in multivariable models remains modest. Therefore, routine genotyping for all patients may not be currently justified. In Vietnam, the implementation of genotype-guided methadone dosing may face practical challenges, including limited access to pharmacogenetic testing, lack of clinical infrastructure, and cost constraints. However, as testing becomes more affordable and local evidence accumulates, targeted genotyping for high-risk subgroups—such as patients with poor treatment response or adverse effects—could be a feasible and impactful strategy to enhance treatment safety and effectiveness.

Several limitations must be acknowledged. Although the study sample reflects the demographic profile of real-world patients in Vietnamese methadone maintenance treatment (MMT) clinics, it was almost exclusively male (99%) and predominantly of Kinh ethnicity (96.9%). This homogeneity may limit the generalizability of our findings to female patients and ethnic minority groups, who may have different genetic backgrounds, methadone metabolism profiles, or treatment responses. Future research should aim to recruit more diverse and gender-balanced cohorts to ensure broader applicability of pharmacogenetic insights. Moreover, the cross-sectional design precludes causal inference and does not allow for assessment of temporal or dose-adjustment dynamics. Although methadone concentrations were measured at steady state and patients were scheduled for blood sampling before their next supervised dose, strict timing at exactly 24 hours was not always feasible due to behavioral unpredictability among this high-risk population. This could introduce some variability in serum concentration data. Additionally, we did not account for clinical and environmental factors such as, concomitant medications (e.g., antibiotics, antifungals),

and smoking status, all of which may influence CYP2B6 enzyme activity regardless of genotype through phenocopy. Lastly, our genetic analysis was limited to selected variants in *CYP2B6* and *OPRM1*; future studies should focus on the combined analysis of multiple genes, which may also contribute to inter-individual variability in methadone pharmacokinetics and pharmacodynamics.

CONCLUSION

In conclusion, this study highlights the influence of specific *CYP2B6* genotypes and SNPs on methadone pharmacokinetics in Vietnamese patients. Although genetic factors account for a portion of the variability in methadone dose requirements and serum concentrations, clinical variables such as actual daily dose remain the most important determinants. Personalized approaches to MMT dosing that integrate both genetic and clinical information may enhance the safety and effectiveness of therapy for opioid dependence.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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