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Research Paper

Association of *ABCB1*, μ-opioid Receptor, and Cytochrome *P450* Genes with Methadone Dose in Iranian Male Addicts Under Methadone Therapy

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ABSTRACT

Introduction: Treatment of opioid use disorders (OUDs) via safe and effective approaches has been investigated for years. Methadone maintenance treatment (MMT) has been considered an effective therapy for opioid addiction. It has been observed that patients with genetic polymorphisms often show variability in the optimal drug dose requirement and treatment schedule. This study aimed to investigate the relationship between single nucleotide polymorphisms (SNPs) in the ATP-binding cassette subfamily B member-1 (*ABCB1*), the mu-opioid receptor (*OPRM1*), and *cytochrome P450* (CYP) genes and methadone dose in patients undergoing MMT in Mazandaran Province, Iran.

Methods: In a cross-sectional study, 216 male MMT patients (20 to 45 years old) who were involved in the treatment program for at least three months were randomly recruited from six MMT clinics in Mazandaran province between 2018 and 2020. Blood samples were taken, DNA was extracted, and SNPs of *CYP2B6 (*$G^{516}T$, $A^{785}G$), *CYP2C19* (-3402C>T), *CYP3A4* (-392A>G), *OPRM* ($A^{118}G$), and *ABCB1* ($C^{3435}T$, $G^{2677}T$, $G^{2677}A$, and $C^{1236}T$) genes were evaluated using polymerase chain reaction-restriction fragment length polymorphism (RFLP).

Results: Our results showed no significant relationship between all the studied genotypes and methadone dose requirements.

Conclusion: The present study, for the first time in the Mazandaran population, reported no significant correlations between methadone dose requirement and different SNPs in the *ABCB1*, *OPRM1*, and *CYP* genes in MMT patients, which is consistent with other studies conducted on the Iranian population.

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Highlights

• No relationship exists between the single nucleotide polymorphisms (SNPs) of the μ-opioid receptor mu 1 ($A^{118}G$) and methadone dose in MMT patients.

• No relationship exists between the SNPs of cytochrome P450 2B6 ($G^{516}T$, $A^{785}G$) and methadone dose in methadone maintenance treatment (MMT) patients.

• The SNP of cytochrome P450 2C19 (-3402C>T) has no relationship with methadone dose in MMT patients.

• No relationship exists between the SNP of cytochrome P450 3A4 (-392A>G) and methadone dose in MMT patients.

• The SNPs of *ABCB1* ($C^{3435}T$, $G^{2677}T$, $G^{2677}A$, and $C^{1236}T$) are not related to the methadone dose in MMT patients.

Plain Language Summary

Opioid use disorder is a serious concern affecting many people. Treatment methods such as methadone therapy can help addicts manage their use. The needed dose of methadone varies based on genetics. This study focused on understanding whether specific genetic variations (single nucleotide polymorphisms) in certain genes can influence the dose of methadone in patients under methadone therapy from northern Iran. Blood samples were collected from 216 male adult addicts with methadone therapy for at least three months. We assessed the genetic variations in genes related to drug metabolism and opioid response. The results showed no significant relationship between the genetic variations and the methadone dose. This means that in male addicts from northern Iran, factors other than the genetic variations might play a bigger role in determining the methadone dosage.

1. Introduction

In Iran, both illegal and legally prescribed opioid consumption is common (Amin-Esmaeili et al., 2016), and it is associated with high levels of opioid use disorder (OUD) and related burden (Amin-Esmaeili et al., 2012; Malekinejad et al., 2015; Rahimi et al., 2020). According to the published reports, Iran has one of the largest populations of opioid users in the world (Rahimi-Movaghar et al., 2018), with an approximation of 2 million individuals suffering from OUD (Noroozi et al., 2022). The most commonly used opioid in Iran is opium, followed by heroin, crack heroin, and prescription opioids, respectively (Amin-Esmaeili et al., 2016). Crack is the street term for a type of illegal narcotic in the Iranian drug market whose main constituent is heroin (Akhgari et al., 2012).

In Iran, methadone maintenance therapy (MMT) is frequently used to treat patients with OUD (Ekhtiari et al., 2020; Momtazi et al., 2015). Methadone, a potent chiral μ-opioid receptor agonist, is also used to treat withdrawal symptoms and manage pain (Ahmad et al., 2018; Bonhomme et al., 2012; Carpenter et al., 2000). The most crucial target associated with the typical effects of opioids, such as morphine and methadone, is the μ-opioid receptor (Wang et al., 2015). Although the receptor is shared, methadone has a lower abuse likelihood, a longer duration of action, and less sedating effects in comparison to morphine or heroin. Based on these characteristics, methadone complications such as tolerance and withdrawal are less described (Davis & Walsh, 2001). These properties make methadone a suitable medication in maintenance programs to treat OUD. However, despite the worldwide clinical indication of methadone, the reactions to this drug may differ considerably between patients, which leads to variations in the amount of methadone consumed from person to person. Thus, a central challenge in MMT is to optimize the medication dosage to have the best efficiency of treatment while facing the fewest side effects (Saloner & Karthikeyan, 2015). Typically, the decision on the dosage of methadone is taken based on factors such as the patient's clinical situation, reaction to the first dose prescribed, and the guidelines approved for the MMT program in that country (Tenegra & Leebold, 2016). Another critical element that could affect methadone dose requirements in a patient is genetic variability (Packiasabapathy et al., 2020). The data have revealed that variations in genes implicated in the pharmacokinetics and pharmacodynamics of methadone are crucial in defining the dose of the medication (Crettol et al., 2006; Fonseca et al., 2010; Hung et al., 2011).

The ATP-binding cassette subfamily B member-1 (*ABCB1*) gene is an environment-susceptible gene that codes P-glycoprotein, which is not only associated with multidrug resistance and the efficacy of many drugs but also functions as a drug-transport pump at the blood-brain barrier (Beer et al., 2013; Finch & Pillans, 2014). Several reports have demonstrated that the DNA changes of the *ABCB1* gene are linked to the alterations of P-gp function (Fung & Gottesman, 2009; Hoffmeyer et al., 2000; Sia et al., 2010). It has been widely investigated that the single nucleotide polymorphisms (SNPs) of the *ABCB1* gene are involved in pain perception. In addition, it has been reported that *ABCB1* gene SNPs play a critical role in methadone dosage requirements in patients (Beer et al., 2013; Coller et al., 2006; Levran et al., 2008). Moreover, evidence indicates that the genetic polymorphisms of the gene modulate methadone dose, metabolism, and response (Dennis et al., 2014).

The *OPRM1* gene encodes the mu-opioid receptor, which is extensively expressed in the brain. A famous variation described in this gene is the rs1799971 ($A^{118}G$) polymorphism in exon 1, leading to an amino acid change from asparagine to aspartate (Taqi et al., 2019). Studies have shown that the $A^{118}G$ polymorphism is involved in OUDs through decreased receptor expression and signaling efficiency (Beer et al., 2013; Wang et al., 2015). OPRM1 is the primary site of action of methadone as an agonist of the mu-opioid receptor, and studies have claimed that the *OPRM1* variant $A^{118}G$ could affect the patient's response to methadone treatment (Barratt et al., 2012).

Methadone is processed by cytochrome P450 (CYP) through first-pass hepatic metabolism (Taha Ahmad et al., 2018). CYP consists of multiple isomers encoded by *P450* genes, including *CYP3A4*, *CYP2B6*, *CYP2D6*, *CYP2C19*, etc. (Chang et al., 2011). Race and ethnic background have been recognized as significant factors in the prevalence of variant alleles coding for CYP families among the population (Marwa et al., 2014). Previous studies have reported that *CYP3A4*, *CYP2B6*, and *CYP2C19* are among the most critical *CYPs* involved in methadone metabolism and dose requirements (Chen, et al., 2011; Crettol et al., 2005; Gadel et al., 2015; Wang et al., 2013). In this setting, there is strong evidence linking the variation in methadone response and dose requirements with CYP polymorphisms (Ahmad et al., 2018; Crettol et al., 2006; Kharasch & Stubbert, 2013; Shinderman et al., 2003).

Based on reports, the challenge of adjusting the methadone dose is the primary reason for failure in the MMT program in the first year of treatment (Lo et al., 2018). In this regard, due to the limited evidence of the effect of gene polymorphisms on methadone treatment outcomes in Iranian populations, we aimed to investigate the relationship between the appropriate methadone dose in patients involved in the MMT program and polymorphisms of genes of *CYP3A4* (−392A>G), *CYP2C19* (-3402C>T), *CYP2B6* ($G^{516}T$ and $A^{785}G$), *OPRM1* ($A^{118}G$), and *ABCB1* ($C^{343}5T$, $G^{2677}T$, $G^{2677}A$, and $C^{1236}T$) in Mazandaran Province, Iran.

2. Materials and Methods

Study subjects

In a cross-sectional and multi-centric study, 216 cases out of 521 approaching patients on MMT who were confirmed to have a previous OUD in Mazandaran Province in Iran were defined as eligible to be enrolled in the research. The participants were randomly recruited from six MMT clinics in Mazandaran Province between 2018 and 2020. Although the samples were randomly chosen, each center was assigned a subsample proportional to the total number of patients in the MMT. Methadone was administered to patients in the form of syrup, tablets, or both. According to the previous research (Akbari et al., 2021), the adequate daily oral dose of methadone taken by subjects was classified as low (≤40 mg/d), medium (41-109 mg/d), and high (≥110 mg/d). To be eligible for enrollment in the study, patients had to be male, aged 20 to 45 years old, and have been in the MMT program for at least three months. Participants with a medical history and patients who took antivirals, antipsychotics, or other drugs that interfered with the MMT package were excluded from the study. In addition, patients using medications that interfered with the function of CYPs, such as fluconazole, rifampin, carbamazepine, chloramphenicol, phenobarbital, primidone, cimetidine, phenytoin, and isoniazid, were prevented from enrollment in the study.

Study questionnaire

Before the tests, the opioid dosage adequacy scale (ODAS) questionnaire was filled out to confirm that the patients were receiving stable, adequate doses of methadone (González-Saiz, 2004). The ODAS has been known as a suitable measurement tool to evaluate the dose adequacy of methadone medication in MMT programs. The questionnaire comprises six dimensions and covers the areas of heroin consumption, narcotic blockade, subjective and objective opiate withdrawal symp-

Table 1. Characteristics of SNPs, primer sequences, and restriction enzymes used in the study

Gene	Symbol	SNP	Primer Sequences	Enzyme
ABCB1	A	C3435T	F: 5′- TTGATGGCAAAGAAATAAAGC -3′ R: 5′- CTTACATTAGGCAGTGACTCG -3′	MboI
	B1	G2677T	F: 5′- TGC AGG CTA TAG GTT CCA GG -3′ R [T]: 5′- TTT AGT TTG ACT CAC CTT CCC G -3′	BanI BsrI
	B2	G2677A	R [A]: 5′- GTT TGA CTC ACC TTC CCA G -3′	
	C	C1236T	F: 5′- TAT CCT GTG TCT GTG AAT TGC C -3′ R: 5′- CCT GAC TCA CCA CAC CAA TG -3′	HaeIII
OPRM1	D	A118G	F: 5′- GTCTCGGTGCTCCTGGC TACCTCGC -3′ R: 5′- TTCGGACCGCATGGGTC GGACCGGT -3′	Bsh1285I
CYP2B6	E	G516T	F: 5′- GGTCTGCCCATCTATAAAC -3′ R: 5′- CTGATTCTTCACATGTCTGCG -3′	BsrI (BseNI)
	F	A785G	F: 5′- GACAGAAGGATGAGGGAGGAA -3′ R: 5′- CTCCCTCTGTCTTTCATTCTGT -3′	Sty I
CYP2C19	G	-3402C>T	F: 5′- TCA AAA GAT ATA TCT GAT AAATGATGG -3′ R: 5′- ACT GTC TCC TGA AGT GTC TGT AC -3′	MnlI
CYP3A4	H	−392A>G	F: 5′- GGAATGAGGACAGCCATAGAGACAAGGGGA -3′ R: 5′- CCTTTCAGCTCTGTGTTGCTCTTTGCTG -3′	MboII

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toms, craving for heroin, and overmedication. Scores are based on the Likert scale and range between 1 (worst) and 5 (best) (Walcher et al., 2016).

In addition, a brief history was taken to determine the patients' demographics, overall health state, and use of medications.

Genotyping

The patients' venous blood samples (4 mL) were collected 24 hours after the last oral methadone consumption. STAR Blood DNA Extraction Kit (Arian Gene Gostar, Iran) was used to isolate DNA from blood samples, and the extracted materials were kept at -20 ˚C. Polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) was performed to determine sample SNPs. Also, the variants were confirmed via the sequencing technique. Following the PCR product validation, the fragment digestion was performed using restriction enzymes, and the results were visualized by electrophoresis. The primer sequences and restriction enzymes used in the study are listed in Table 1.

Statistical analysis

Statistical analysis was performed by the SPSS, software, version 24 (Armonk, NY: IBM Corp.). The chi-square and Fisher exact tests were used to compare the variation frequencies of studied SNPs in three groups of subjects. The results were considered significant with a $P<0.05$.

3. Results

We included only male subjects in the study to avoid the possible effects of gender on the results. The sample size in this research consisted of 216 male MMT subjects, including 22 individuals with the age range of 20-25(10%), 37 with the age range of 26-30(17%), 52 with the age range of 31-35(24%), 56 with the age range of 36-40(26%), and 49 with the age range of 41-45(23%) years.

Table 2. Demographic variables of subjects

Group		No. (%)
Age (y)	20-25	22(10)
	26-30	37(17)
	31-35	52(24)
	36-40	56(26)
	41-45	49(23)
Total		216(100)
Methadone form	Syrup	179(82.87)
	Tablets	23(10.65)
	Syrup & tablets	14(6.48)
Consumption dose (mg/day)	Low (≤40)	58(26.8)
	Medium (41-1090)	143(66.2)
	High (≥110)	15(7)

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The medication consumption of study subjects was as follows: 82.87% (179 individuals) received methadone in the form of syrup (70.28±28.37 mg/d), 10.65% (23 individuals) in the form of a tablet (70.16±22.12 mg/d), and 6.48% (14 individuals) in both tablet and syrup forms (71.92±20.56 mg/d). Patients received methadone in a mean dosage of 69.63±27.59 mg/d (15-200 mg/kg). The mean length of MMT in patients was 33.7 months. It should be mentioned that the patients enrolled in the study were all in the stable maintenance phase of MMT, and those subjects willing to decrease or discontinue methadone were not included in the study. The classification of patients based on their consumed dose of methadone and the number of subjects in each group is shown in Table 2.

The frequency and percentage of SNPs in the study subjects have been shown in detail in Table 3. The distribution of genotypes in the study groups was evaluated via chi-square statistics and found to be in Hardy-Weinberg equilibrium for variations A (P=0.73), B1 (P=0.06), C (P=0.06), and H (P=0.65), but not in equilibrium for B2 (P<0.001), D (P<0.001), E (P<0.05), F (P<0.01), and G (P<0.05).

In addition, as shown in Table 4, there were no significant differences (P>0.05) in SNP frequencies of studied genes between three categories of low-, moderate-, and high-dose methadone consumers.

4. Discussion

The primary purpose of this study was to investigate the association of *ABCB1*, *OPRM1*, and *CYP* genetic variations with the methadone dose in MMT patients in Mazandaran, Iran. Based on the findings, we may conclude that there is no association between the studied genetic polymorphisms and the dosage of methadone intake in this context.

CYP3A4 is extensively involved in methadone metabolism, as are, to a lesser extent, *CYP2B6* and *CYP2C19* (Kapur et al., 2011). Despite the prominent role of *CYP* in the metabolism of methadone, limited investigations have been conducted to verify the influence of its genetic variations on MMT results. In this setting, Chen and colleagues have reported that patients carrying the allele type *CYP3A4*1B* demonstrate higher methadone plasma levels. Furthermore, the increase in methadone plasma level confirms the variations in methadone requirement levels in that studied population (Chen, et al., 2011). Regarding our findings, no significant relationship was detected between the studied genotypes and allele types of *CYP3A4*, *CYP2B6*, and *CYP2C19* and methadone dose requirement. In agreement with our results, Akbari and colleagues have reported no significant association between the *CYP3A4*1B* variant and methadone dose in Iranian patients undergoing the MMT program (Akbari et al., 2021).

Table 3. The frequency and percentage of SNPs in study subjects

SNP Symbol*	No. (%)		
	-/-	+/-	+/+
A	48(22.2)	105(48.6)	63(29.2)
B1	39(18)	121(56)	56(26)
B2	204(94.4)	9(4.2)	3(1.4)
C	17(7.85)	68(31.5)	131(60.65)
D	148(68.5)	27(12.5)	41(19)
E	11(5)	54(25)	151(70)
F	42(19.5)	84(38.9)	90(41.6)
G	14(6.5)	60(27.8)	142(65.7)
H	0(0)	13(6)	203(94)

*The symbols are defined in Table 1.

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Table 4. Frequency of studied genotypes in low, medium, and high dose methadone consumers

The Symbol of Variation*	Genotype	Methadone Consumption Dosage				P
		Total	Low	Medium	High	
A	-/-	48	14	32	2	0.245
	-/+	105	23	71	11	
	+/+	63	21	40	2	
	Total	216	58	143	15	
B1	-/-	39	9	28	2	0.68
	-/+	121	30	81	10	
	+/+	56	19	34	3	
	Total	216	58	143	15	
B2	-/-	204	52	138	14	0.183
	-/+	9	4	4	1	
	+/+	3	2	1	0	
	Total	216	58	143	15	
C	-/-	17	4	12	1	0.97
	-/+	68	17	47	4	
	+/+	131	37	84	10	
	Total	216	58	143	15	

The Symbol of Variation*	Genotype	Methadone Consumption Dosage				P
		Total	Low	Medium	High	
D	-/-	148	44	93	11	0.349
	-/+	27	6	18	3	
	+/+	41	8	32	1	
	Total	216	58	143	15	
E	-/-	11	3	8	0	0.38
	-/+	54	15	32	7	
	+/+	151	40	103	8	
	Total	216	58	143	15	
F	-/-	42	10	28	4	0.713
	-/+	84	21	56	7	
	+/+	90	27	59	4	
	Total	216	58	143	15	
G	-/-	14	3	11	0	0.838
	-/+	60	18	37	5	
	+/+	142	37	95	10	
	Total	216	58	143	15	
H	-/-	0	0	0	0	1.00
	-/+	13	3	9	1	
	+/+	203	55	134	14	
	Total	216	58	143	15	

*The symbols are described in Table 1.

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The μ-opioid receptor is a promising target in opioid consumption. It is claimed that the enantiomers of methadone differentially exert their effects via the activation of μ-opioid receptors (Matsui & Williams, 2010). In this setting, numerous studies have focused on the association of polymorphisms of the μ-opioid receptor (*OPRM1*) gene with the treatment response of methadone. However, the conflicting results in the literature regarding any correlation between μ polymorphism and MMT response have been challenging. For example, the SNP rs558025 was reported to have an association with MMT outcome (Levran et al., 2013), while the studies done by two other groups of researchers could not show a correlation between μ polymorphism and MMT results or dosage of methadone (Crettol et al., 2008; Hung et al., 2011). In agreement with the latter, our results revealed that the $A^{118}G$ polymorphism of the *OPRM1* gene had no significant association with methadone intake. In line with this finding, Beer and colleagues found no significant correlation between $A^{118}G$ genotyping and drug abuse in the European population (Beer et al., 2013).

It is well known that P-gp expression and function are strongly linked to *ABCB1* gene variations. Therefore, any functional impairment in P-gp could be a possible consequence of the *ABCB1* gene polymorphism (Jiang et al., 2019; Lin, 2020). It is well established that the *ABCB1* multi-locus genotype is a critical factor in the outcome of the MMT program and opioid dosage requirements (Beer

et al., 2013; Parchure & Peng, 2020). Based on a previous report, the *ABCB1* gene SNPs, including rs1045642, rs2032582, and rs1128503, could significantly affect the chance of requiring a higher or lower dose of methadone in people undergoing an MMT program who were previously involved in heroin use disorder (Levran et al., 2008). In line with these findings, further studies have confirmed the prominent role of *ABCB1* gene polymorphisms in opioid dosage requirements (Coller et al., 2006; Horvat et al., 2017; Hung et al., 2011; Luo et al., 2017). On the other hand, some other investigations have reported no significant correlation between the methadone dose requirement and *ABCB1* polymorphisms (Crettol et al., 2006; Fonseca et al., 2011; Hamraz et al., 2019). In agreement with the latter studies, our results also revealed that the most frequently occurring *ABCB1* multi-locus genotype patterns, such as $C^{3435}T$, $G^{2677}A$, and $C^{1236}T$, did not demonstrate any significant linkage to the dose of consumed methadone in 216 MMT patients.

5. Conclusion

Our results indicated no potential contribution of ABCB1, CYP, and OPRM1 genes variations to the variability in methadone dosage in the MMT population. The results of this study and previous investigations suggest that the effect of genetic polymorphisms on OUD development and maintenance treatment outcomes needs to be further investigated in future studies. In addition, regarding the current study's findings, it would be informative if correlations between demographic characteristics (e.g. age, education), length of MMT, genetic variables, and dosing groups were evaluated in subsequent reports. Furthermore, it would be helpful to use other categorizations, such as patients receiving very high maintenance doses (above 150 mg/day) with the different groups in the subsequent studies.

Ethical Considerations

Compliance with ethical guidelines

The study protocols were approved by the Ethics Committee of the Tehran University of Medical Sciences, Tehran, Iran (Code: IR.TUMS.REC.1394.172). All the volunteers were fully informed about the study objectives and methods and gave informed written consent.

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Authors' contributions

Conceptualization and supervision: Nasim Vousooghi, Mohammad Reza Zarrindast, and Anahita Torkaman-Boutorabi; Methodology: Nasim Vousooghi, Emran Mohammad Razaghi, and Mohammad Reza Mahdavi; Data collection and data: Abdollah Golnezhad and Mohammad Reza Mahdavi; Investigation and writing the original draft: Abdollah Golnezhad; Review, editing, funding acquisition and project administration: Nasim Vousooghi.

Conflict of interest

The authors declared no conflict of interest.

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