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ABCB1, *CYP2B6*, and *CYP3A4* genetic polymorphisms do not affect methadone maintenance treatment in HCV-positive patients

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The aim of this study was to determine the influence of *ABCB1*, *CYP2B6,* and *CYP3A4* genetic polymorphisms on methadone metabolism in patients with hepatitis C virus (HCV) undergoing methadone maintenance treatment (MMT). The study included 35 participants undergoing MMT, who were divided in three groups: HCV-positive (N=12), HCV-negative (N=16), and HCV clinical remission (CR) (N=7). The concentrations of methadone and its main metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) were determined with gas chromatography-mass spectrometry. The patients were genotyped for *ABCB1* rs1045642, *CYP2B6* rs3745274, *CYP3A4* rs2242480, and *CYP3A4* rs2740574 polymorphisms. Differences between single nucleotide polymorphism (SNP) genotypes and methadone-to-EDDP ratio were analysed with one-way ANOVA, which showed no significant difference between the genes (p=0.3772 for *ABCB1* rs1045642, p=0.6909 for *CYP2B6* rs3745274, and p=0.6533 for *CYP3A4* rs2242480). None of the four analysed SNP genotypes correlated with methadone-to-EDDP concentration ratio. A major influence on it in hepatitis C-positive patients turned out to be the stage of liver damage.

KEY WORDS: EDDP; genotyping; hepatitis C; liver damage; SNP

Methadone is a synthetic opioid used for detoxification and maintenance treatment of patients who are dependent on opiates, mainly heroin. In the liver it is metabolised to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) through *N*-demethylation mediated by several cytochrome P450 (CYP) enzymes, including CYP3A4 and CYP2B6 (1). The CYP3A4 enzyme metabolises both (*R*)- and (*S*)-methadone enantiomers, but the (*S*)-enantiomer is mostly metabolised by CYP2B6 (2, 3).

Methadone kinetics is also slightly affected by *ABCB1* genetic polymorphisms (4). In a study of their effects on methadone maintenance treatment (MMT) in 60 opioid-dependent patients, Coller et al. (5) reported that *ABCB1* genetic variability influenced daily methadone requirements.

In addition, *CYP* gene variants may contribute to the risk of fatal methadone toxicity posed by high concentrations of unmetabolised methadone in plasma (6). The risk may be even greater in patients with liver damage caused by hepatitis C virus (HCV) infection, which is common in opioid dependency. Our earlier studies (7, 8) have shown that chronic overdose and liver insufficiency (damage) increase the amount of unmetabolised methadone and its toxicity, but we did not consider the influence of the *ABCB1*, *CYP2B6,* and *CYP3A4* genes on methadone metabolism as one of the numerous factors that influence inter-individual variability in that respect. The aim of this study was therefore to address this gap and see if the *ABCB1*, *CYP2B6*, and *CYP3A4* genetic polymorphisms affect methadone metabolism in patients on MMT.

MATERIALS AND METHODS

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the University Hospital Split, Croatia (No. 530-01/12-01/164). All patients signed informed consent to participation in the study.

Participants

Our study participants were 35 adult men aged ≥21 years, who were in the MMT programme conducted by the Institute for Public Health of the Split-Dalmatia County, Croatia. To arrive to this number we first assessed for

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eligibility 74 adult male heroin addicts in the programme according to the following inclusion criteria: male, Caucasian, at least 9 months on MMT with regular attendance, no HIV or HBV co-infection or infection, negative urine tests for the presence of heroin or other pharmacological substances that could interfere with methadone metabolism, and no liver cirrhosis or history of significant alcohol abuse. HCV-positive patients were receiving interferon therapy that does not interfere with methadone metabolism (9). Thirty-nine candidates who did not meet these criteria were excluded.

The remaining 35 participants were divided in groups according to their HCV status: HCV-negative (HCV-) (N=12), HCV positive (HCV+) (N=16), and those in clinical remission (HCV CR) (N=7). Their liver damage was assessed according to the fibrosis-4 (FIB-4) index as described elsewhere (10). They were receiving different recommended doses of oral methadone based on their clinical presentation, and these doses were not modified for at least six months before the study (Table 1).

Methadone and EDDP determination

For this study, methadone and EDDP concentrations were tested only in urine during three regular check-ups 15 days apart. We took two urine samples per check-up; one immediately before and the other 90 min after oral methadone administration, which totalled six samples per participant. The samples were stored at 4 °C and analysed within 1–4 days as described in detail in our previous reports (8, 11). Methadone and EDDP concentrations were used to calculate their ratio (Table 1).

DNA analysis

Blood samples for DNA analysis were collected at regular check-ups and stored in EDTA tubes. DNA was isolated with a commercial genomic DNA isolation kit (High Pure PCR Template Preparation Kit, Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. Extracted DNA was quantified using a Qubit 4 fluorimeter (Thermo Fischer Scientific, Waltham, MA, USA).

SNP genotyping

Using the TaqMan® SNP genotyping assay (Thermo Fischer Scientific) and an Applied Biosystems 7500 real-time polymerase chain reaction (RT-PCR) system (Applied Biosystems, Foster City, CA, USA) we genotyped for the following single-nucleotide polymorphisms (SNPs): *ABCB1* rs1045642 (DME C_7586657_20), *CYP2B6* SNP rs3745274 (DME C_7817765_60), *CYP3A4* rs2242480 (DME C_26201900_30), and *CYP3A4* rs2740574 (DME C_1837671_50). RT-PCR and allelic discrimination analyses were performed according to the manufacturer's instructions in a 25-mL reaction volume. The temperature program for RT-PCR was 60 °C for 1 min and 95 °C for 10 min, followed by 50 cycles of 92 °C for 15 s and 60 °C for 90 s. SNP genotypes were determined using instrument software with the manual allele call option.

Statistical analysis

Statistics and differences between the samples and groups were tested by using GraphPad Prism version 8.0.0 for Mac (GraphPad Software, San Diego, CA, USA). Hardy-Weinberg equilibrium, chi-square, and the *p* value were calculated with an on-line calculator (12).

RESULTS AND DISCUSSION

Methadone is converted to its inactive metabolites EDDP and EMDP by hepatic CYP450 enzymes. Its pharmacokinetics is individual, not only because of differences in sex, age, body weight, and use of other drugs, but also because of different allelic frequencies of genetic polymorphisms (2). Some earlier studies have already shown that allelic variations in the *ABCB1* rs1045642, *CYP2B6* rs3745274, and *CYP3A4* rs2242480 and rs2740574 SNPs may affect the distribution of methadone in patients undergoing MMT (6, 13, 14).

Table 2 shows the genotypes of the four investigated SNP loci in DNA obtained from blood samples. Genotype frequencies were consistent with the Hardy-Weinberg equilibrium and did not significantly differ for any of the studied SNPs (the *p* value ranged from 0.55 to 0.93). Minor allele frequencies (MAF) (Table 2) were consistent with those presented in a meta study by Dennis et al. (15), in which they ranged between 24.5 and 50 % for *ABCB1* rs1045642 and between 24 and 39 % for *CYP2B6* rs3745274.

In contrast to *ABCB1* and *CYP2B6* SNPs, no mutated alleles were found in either of the analysed *CYP3A4* SNPs (Figure 1).

We observed a small difference in wild and mutant genotypes between the *ABCB1* and *CYP2B6* genes. Patients with the *ABCB1* AA and *CYP2B6* TT genotype had a slower

Table 1 Average FIB-4 index, methadone-to-EDDP urine concentration ratio, and methadone dose received by study groups (N=35)

Parameters	HCV-negative (n=12)	HCV-positive (n=16)	HCV clinical remission (n=7)
FIB-4 index	0.81	2.61	2.08
Methadone-to-EDDP urine concentration ratio	0.87	2.04	0.79
Methadone dose (mg)	83.2	85.0	62.5

Table 2 Genotype and allele frequencies (%) by loci for the *ABCB1*, *CYP2B6* and *CYP3A4* genes in all participants (N=35)

Gene	SNP	Genotype	n (%)	Minor allele	MAF (%)	Hardy-Weinberg equilibrium	
						p allele frequency	χ^2 p-value
ABCB1	rs1045642	**GG**	12 (34.3)	**A**	42.9	0.57	0.15 0.69
		AG	16 (45.7)				
		AA	7 (20)				
CYP2B6	rs3745274	**GG**	15 (42.9)	**T**	32.9	0.67	0.36 0.55
		GT	17 (48.5)				
		TT	3 (8.6)				
CYP3A4	rs2242480	**CC**	30 (85.7)	**T**	7.1	0.93	0.21 0.65
		CT	5 (14.3)				
		TT	0 (0)				
CYP3A4	rs2740574	**TT**	34 (97.1)	**C**	1.4	0.99	0.007 0.93
		CT	1 (2.9)				
		CC	0 (0)				

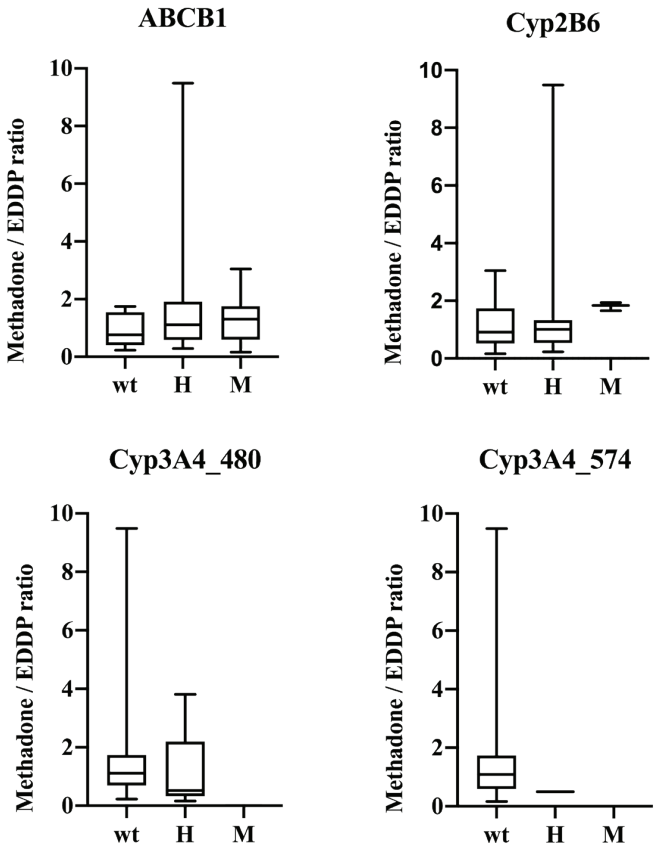

Figure 1 Methadone-to-EDDP ratio in all patients with different *ABCB1, CYP2B6, CYP3A4* 480 and *CYP3A4*_574 variants. H – heterozygotes; M – mutant; wt – wild type

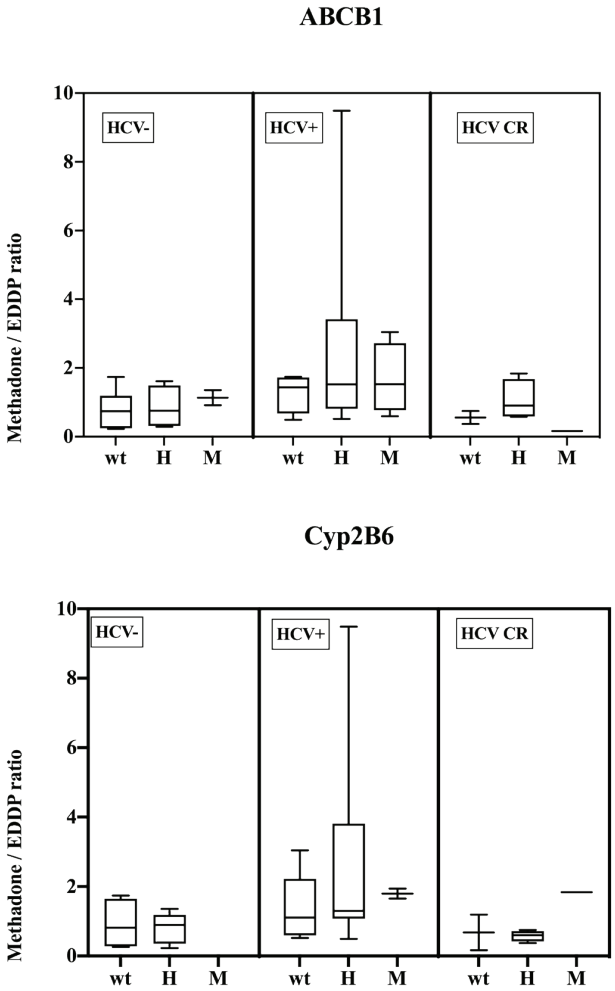

Figure 2 Methadone-to-EDDP ratio in patients with different *ABCB1*and *CYP2B6* variants by HCV status. H – heterozygotes; HCV- – HCV-negative group; HCV+ – HCV-positive group; HCV CR – group in HCV clinical remission ; M – mutant; wt – wild type

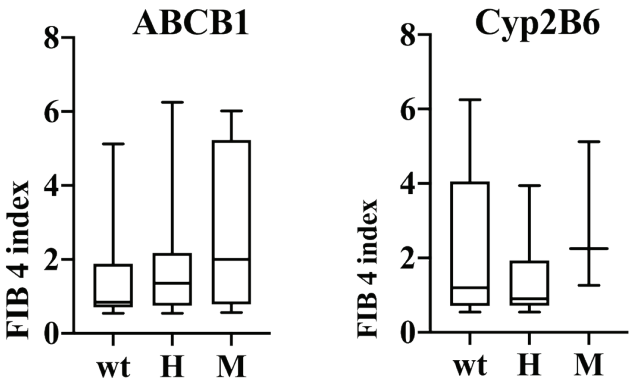

Figure 3 FIB-4 index in all patients with different *ABCB1* and *CYP2B6* variants. H – heterozygotes; M – mutant; wt – wild type

methadone metabolism than those with the GG genotype. In both SNPs of the *CYP3A4* gene the wild genotype had a slower methadone metabolism than the heterozygous genotype.

Although several studies have suggested an association between genetic variability and methadone metabolism, our results showed no significant difference in methadone-to-EDDP ratio between the SNP genotypes. For *ABCB1* rs1045642 it was p=0.3772 (ANOVA F=1.005), for *CYP2B6* rs3745274 p=0.6909 (F=0.374), and for *CYP3A4* rs2242480 p=0.6533 (F=0.4313).

Even within the groups divided by HCV status this difference was not significant (p>0.05) (Figure 2). In the HCV+ group, the methadone-to-EDDP ratio was similar for all three *ABCB1* genotypes and slightly higher in patients with the mutated *CYP2B6* genotype.

The liver is the primary target organ of HCV infection and is the main organ responsible for metabolism of drugs. Wu et al. (16) reported that HCV affected methadone metabolism in MMT patients. Our results seem to confirm this finding, as the severity of liver damage (median FIB-4 index) was the highest in HCV patients, who also showed the slowest methadone metabolism (the highest methadone-to-EDDP ratio; Table 1).

Figure 3 shows FIB-4 indices relative to the *ABCB1* and *CYP2B6* gene polymorphisms for all 35 patients. The median FIB-4 index in both examined genes was higher in patients with the mutated genotype. However, we found no statistically significant difference in FIB-4 index between the SNP genotypes. For *ABCB1* rs1045642 it was p=0.4465 (ANOVA F=0.8269) and for *CYP2B6* rs3745274 p=0.1551 (F=1.977).

In conclusion, our study found no significant influence of the established genetic polymorphisms in our patients with methadone metabolism. Similar has been reported by Fonseca et. al. (17), who found negligible impact of the *CYP3A5, CYP2B6, CYP2C9, CYP2C19*, and *ABCB1* genetic polymorphisms on *S*-methadone metabolite plasma concentrations (16).

The only major influence on the methadone-to-EDDP ratio in HCV+ patients we found was the stage of liver damage. HCV- and HCV+ patients were taking similar methadone doses. The FIB-4 index and the methadone-to-EDDP ratio in HCV- patients were lower, regardless of the genotype.

However, our conclusion should be taken with reserve, as this study had a small sample size. Future research should therefore involve more MMT patients.

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Polimorfizmi gena *ABCB1, CYP2B6* i *CYP3A4* ne utječu na metadonsku terapiju održavanja u bolesnika s HCV-om

Cilj ovoga istraživanja bio je utvrditi utjecaj polimorfizama gena *ABCB1, CYP2B6 i CYP3A4* na metabolizam metadona u bolesnika/ovisnika s pozitivnim nalazom virusa hepatitisa C (HCV) na metadonskoj terapiji održavanja. Istraživanje je provedeno na uzorku od 35 sudionika na metadonskoj terapiji održavanja, podijeljenih u sljedeće skupine: HCV pozitivni (N=12), HCV negativni (N=16) i oni s kliničkom remisijom HCV-a (CR) (N=7). Koncentracije metadona i njegova glavnog metabolita 2-etiliden-1,5-dimetil-3,3-difenilpirolidina (EDDP) utvrđene su plinskom kromatografijom – masenom spektrometrijom. U sudionika su analizirani genski polimorfizmi *ABCB1* rs1045642, *CYP2B6* rs3745274, *CYP3A4* rs2242480 i *CYP3A4* rs2740574. Jednosmjerna analiza varijance (engl. *one-way ANOVA*) nije pokazala statistički značajne razlike između genotipova jednonukleotidnih polimorfizama (engl. *single-nucleotide polymorphism*, krat. SNP) u omjeru koncentracije metadona i EDDP-a (p=0,3772 za *ABCB1* rs1045642; p=0,6909 za *CYP2B6* rs3745274 F=0,374 i p=0,6533 za *CYP3A4* rs2242480). Nijedan od četiriju analiziranih SNP genotipova nije korelirao s omjerom koncentracije metadona i EDDP-a. U bolesnika/ovisnika koji su bili pozitivni na HCV na taj je omjer ponajviše utjecao stupanj oštećenja jetre.

KLJUČNE RIJEČI: EDDP; genotipiziranje; hepatitis C; jednonukleotidni polimorfizmi; oštećenje jetre; SNP