CYP3A5 POLYMORPHISM IN SERBIAN PAEDIATRIC EPILEPTIC PATIENTS ON CARBAMAZEPINE TREATMENT

Dragana Dragas Milovanovic[1], Ivan Radosavljevic[2], Marija Radovanovic[3], Jasmina R. Milovanovic[1], Slobodan Obradovic[3], Slobodan Jankovic[1], Dragan Milovanovic[1] and Natasa Djordjevic[1]

[1] Department of Pharmacology and Toxicology, Faculty of Medical Sciences, University of Kragujevac, Serbia;
[2]Department of Surgery, Faculty of Medical Sciences, University of Kragujevac, Serbia;
[3]Department of Paediatrics, Faculty of Medical Sciences, University of Kragujevac, Serbia

POLIMORFIZAM CYP3A5 KOD DECE SA EPILEPSIJOM LEČENE KARBAMAZEPINOM U SRBIJI

Dragana Dragaš Milovanović[1], Ivan Radosavljević[2], Marija Radovanović[3], Jasmina R. Milovanović[1], Slobodan Obradović[3], Slobodan Janković[1], Dragan Milovanović[1] i Nataša Đorđević[1]

[1] Katedra za farmakologiju i toksikologiju, Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Srbija
[2]Katedra za hirurgiju, Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Srbija
[3]Katedra za pedijatriju, Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Srbija

Received / Primljen: 03. 11. 2014. Accepted / Prihvaćen: 23. 04. 2015.

ABSTRACT

*Carbamazepine exhibits significant inter-individual variability in its efficacy and safety, which leads to unpredictable therapy outcomes for the majority of patients. Although its complex biotransformation depends on CYP3A5 activity, evidence of association between carbamazepine treatment outcomes and CYP3A5 functional variations remains inconclusive. The aim of the present study was to investigate the distribution of two of the functionally important CYP3A5 variants *2 and *3 as well as their effects on carbamazepine dose requirements, plasma concentrations and clearance in a Serbian population. The study involved 40 paediatric epileptic patients on steady-state carbamazepine treatment. Genotyping was conducted using the PCR-RFLP method, and carbamazepine plasma concentrations were determined using the HPLC method. CYP3A5*2 and *3 polymorphisms were found at frequencies of 0.0% and 97.5%, respectively, which corresponds well to previously published data for Caucasians. No differences in CYP3A5*3 allele frequencies were detected among epileptic patients in comparison to healthy volunteers within similar ethnic populations (p>0.08), indicating that CYP3A5 polymorphism does not represent a risk factor for epilepsy development. There was an observed tendency towards lower dosage requirements (mean±SD: 15.06±4.45 mg/kg vs. 18.74±5.55 mg/kg; p=0.26), higher plasma concentrations (mean±SD: 0.45±0.13 mg/kg vs. 0.38±0.03 mg/kg; p=0.47) and lower clearance (mean±SD: 0.14±0.05 mg/kg vs. 0.15±0.01 mg/kg; p=0.79) of carbamazepine in homozygous carriers of CYP3A5*3/*3 compared to heterozygous CYP3A5*1A/*3 Serbians. Because these genotype groups did not differ significantly in terms of their carbamazepine pharmacokinetics parameters, the proposed effects of CYP3A5*3 on carbamazepine metabolism could not be confirmed.*

Key words: *CYP3A5 polymorphism, Serbian, epilepsy, carbamazepine*

SAŽETAK

*Karbamazepin odlikuje značajna inter-individualna varijabilnost u efikasnosti i bezbednosti, zbog koje je ishod terapije kod većine pacijenata neizvestan. Iako njegova složena biotransformacija zavisi od aktivnosti CYP3A5 enzima, definitivni dokazi o povezanosti ishoda lečenja karbamazepinom i funkcionalnih varijacija CYP3A5 gena još uvek ne postoje. Cilj ove studije bio je da ispita distribuciju dve funkcionalno značajne varijacije CYP3A5 gena *2 i *3, kao i njihov uticaj na potrebnu dozu, plazma koncentraciju i klirens karbamazepina, u srpskoj populaciji. Studija je uključila 40 pedijatrijskih pacijenata sa epilepsijom lečenih karbamazepinom, nakon postignutog ravnotežnog stanja. Genotipizacija je sprovedena PCR-RFLP, a plazma koncentracija karbamazepina izmerena HPLC metodom. Učestalost CYP3A5*2 i *3 polimorfizama bila je 0.0% i 97.5%, što odgovara prethodno publikovanim podacima za belu populaciju. Nije bilo razlike u učestalosti CYP3A5*3 alela kod pacijenata sa epilepsijom u poređenju sa zdravim ispitanicima iz istih populacija (p>0.08), što ukazuje da CYP3A5 polimorfizam nije faktor rizika za razvoj epilepsije. Uočena je tendencija ka nižim potrebnim dozama (mean±SD: 15.06±4.45 mg/kg naspram 18.74±5.55 mg/kg; p=0.26), višim plazma koncentracijama (mean±SD: 0.45±0.13 mg/kg vs. 0.38±0.03 mg/kg; p=0.47) i nižem klirensu (mean±SD: 0.14±0.05 mg/kg vs. 0.15±0.01 mg/kg; p=0.79) karbamazepina kod homozigotnih CYP3A5*3/*3 u poređenju sa heterozigotnim CYP3A5*1A/*3 genotipovima kod Srba. Obzirom da nije bilo značajne razlike u farmakokinetičkim parametrima karbamazepina među različitim grupama genotipova, predloženi efekat CYP3A5*3 na metabolizam karbamazepina nije mogao biti potvrđen.*

Ključne reči: *CYP3A5 polimorfizam, srpski, epilepsija, karbamazepin*

DE GRUYTER OPEN

UDK: 616.853-085; 615.213 / Ser J Exp Clin Res 2015; 16 (2): 93-99
DOI: DOI: 10.1515/SJECR-2015-0012

Corresponding author: Natasa Djordjevic, MD, PhD, Associate Professor, Department of Pharmacology and Toxicology, Faculty of Medical Sciences, University of Kragujevac, Svetozara Markovica 69, 34 000 Kragujevac, Serbia
Tel: +381 34 306 800, ext 223; E-mail: natashadj2002@yahoo.com

ABBREVIATIONS

CYP – Cytochrome P450;
CYP3A5 – Cytochrome P450 3A5;
EDTA - Ethylene diaminetetracetic acid;
PCR - Polymerase chain reaction;
PCR-RFLP - Polymerase chain reaction - restriction fragment length polymorphism;
HPLC - High-performance liquid chromatography

INTRODUCTION

Epilepsy is a common, widely distributed, neurological disorder, known for its frequent resistance to and serious adverse reactions during treatment (1, 2). Since the introduction of potassium chloride in the 19th century, numerous anticonvulsant drugs have been used in therapy for epilepsy (3). Unfortunately, all of these anticonvulsants have exhibited significant inter-individual variability in their efficacy and safety, which leads to an unpredictable therapy outcome in the majority of patients (4, 5). This can be a consequence of many different factors, including variations in the genes coding for drug metabolizing enzymes, transporters and receptors (6). Nevertheless, the pharmacogenetics of epilepsy are still largely unknown (6).

Carbamazepine is a well-known anticonvulsant, frequently used as the first line therapy in several forms of both adult and childhood epilepsy (7, 8). It undergoes a complex biotransformation that involves several drug metabolizing enzymes, with major routes depending primarily upon CYP2C8 and members of the CYP3A family CYP3A4 and CYP3A5 (7, 9). In contrast with CYP3A4, which contributes to variable drug responses through inducibility rather than polymorphism, CYP3A5 exhibits high genetic variability dependent upon ethnicity, as well as strong associations between its genetic background and drug metabolizing activity (10, 11). The clinical significance of *CYP3A5* polymorphism has been reported for several CYP3A5 substrates, including verapamil, tacrolimus and saquinavir (12-14). However, due to conflicting reports, the evidence of association between carbamazepine treatment outcomes and *CYP3A5* functional variations remains inconclusive. As an example, although *CYP3A5* genotype affected serum concentration of carbamazepine in Koreans (15), Chinese (16), and Japanese (17), as well as drug half-life in African-Americans (18), no influence of carbamazepine pharmacokinetics parameters were observed in Caucasian epileptic patients (18). The apparent underlying cause of this discrepancy might be interethnic variability, which represents a multidimensional determinant that comprises both genetic heritage and environment (19, 20). Thus, additional studies on other ethnic populations could contribute to a better understanding of inter-individual differences in carbamazepine response. To our knowledge, no similar investigation has been conducted in Serbian epileptic patients to date.

With an aim to explore the potential role of *CYP3A5* genetic polymorphisms in carbamazepine metabolism, we investigated the distribution of two of the functionally important *CYP3A5* variants and their effects on drug dosage requirements, plasma concentrations and clearance in Serbian epileptic patients undergoing carbamazepine treatment. As the relative risk of adverse drug reactions in children is known to be several-folds higher than in adults (21), only a paediatric population was included in the study.

MATERIALS AND METHODS

Study subjects

The study involved 40 Serbian epileptic patients on steady-state carbamazepine treatment (Table 1). The subjects were recruited from the paediatric department of the Clinical Centre, Kragujevac, Serbia. To be enrolled in the study, all patients had to meet the following inclusion criteria: 1) age between 2 and 20 years, 2) diagnosed partial or generalized tonic-clonic seizures, 3) ongoing carbamazepine treatment, and 4) Serbian origin. The exclusion criteria were as follows: 1) presence of known contraindications for carbamazepine; 2) use of grapefruit juice; 3) presence of atrioventricular block, suppression of bone marrow or porphyria; 4) diagnosed absence or myoclonic epilepsy; 5) presence of increased intraocular pressure; and 6) pregnancy or breastfeeding. Of the 40 enrolled patients, four were co-treated with valproate, whereas others were on carbamazepine monotherapy. Written informed consent was obtained from all patients and their parents, and the study was approved by the ethics committee at the Clinical Centre, Kragujevac, Serbia. The study was conducted in accordance with the Declaration of Helsinki and its subsequent revisions.

Table 1: Patient characteristics

Number of patients	40
Gender (male/female)	24/16
Total body weight (kg)	mean ± SD: 40.38±12.82; range: 17-65
Age (years)	mean ± SD: 10.58±2.88; range: 4-20
Epilepsy (idiopathic/symptomatic)	34/6

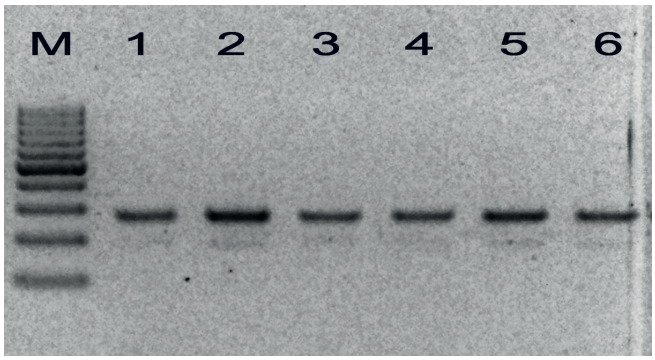

Figure 1. PCR-RFLP analysis of *CYP3A5*2* allele.
Lane M: 100 bp DNA ladder; lanes 1-6: 27289C/C genotype

Genotyping and drug analysis

DNA was extracted from whole-blood samples with EDTA using the PurelinkTM genomic DNA kit (Invitrogen, Carlsbad, CA). DNA concentration was measured using a Qubit® 2.0 Fluorometer and Qubit™ dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA).

*CYP3A5*2* (27289C>A, rs28365083) was genotyped using the PCR-RFLP method described by van Schaik et al. (22), with minor modifications. In brief, a 269-bp-long *CYP3A5* region of interest was amplified in 20 µl PCR of reaction mixture, consisting of ~20 ng of DNA, 0.2 µM dNTP Mix (Thermo Scientific, Waltham, MA), 1.7 mM $MgCl_2$, 0.2 µl of primers 5'-CTGTTTCTTTCCTTCCAGGC-3' and 5'-CTCCATTTCCCTGGAGACTTG-3' (Invitrogen, Carlsbad, CA) and 0.5 U DreamTaqDNA Polymerase (Thermo Scientific, Waltham, MA), in 1XPCR buffer (Qiagen, Hilden, Germany). The amplification was conducted under the following conditions: initial denaturation at 94°C for 7 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 70°C for 1 min; and final extension at 72°C for 7 min. PCR products were subjected to the restriction enzyme FastDigest® Tsp509I (Thermo Scientific, Waltham, MA), which cuts only variant-type alleles to fragments of 182 bp and 87 bp (Fig. 1).

Genotyping for *CYP3A5*3* (6986A>G, rs776746) was performed according to King et al. (23). Namely, PCR yielded 196-bp-long amplicons in a 15 µl mixture containing ~20 ng of DNA, 1XPCR buffer (Qiagen, Hilden, Germany), 0.2 µM dNTP Mix (Thermo Scientific, Waltham, MA), 2.5 mM MgCl2, 0.2 µl of primers 5'-CTGTTTCTTTCCTTCCAGGC-3' and 5'-CTCCATTTCCCTGGAGACTTG-3' (Invitrogen, Carlsbad, CA) and 0.5 U DreamTaqDNA Polymerase (Thermo Scientific, Waltham, MA). The conditions of the PCR reaction were as follows: initial denaturation at 94°C for 2 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 61°C for 1 min, extension at 70°C for 1 min; final extension at 72°C for 7 min. Restriction digestion at 37°C with FastDigest® RsaI enzyme (Thermo Scientific, Waltham, MA) resulted in cutting wild type alleles to 102-bp, 94-bp, 74-bp and 20-bp fragments, and variant type alleles to 102-bp, 74-bp and 20-bp fragments (Fig. 2).

Both PCR reactions were conducted in Techne Genius PCR Thermal Cyclers (Techne, Cambridge, UK). The PCR products and restriction fragments were detected by gel electrophoresis on a 1.2% or 2.4% agarose gel stained with Sybr® safe DNA gel stain (Invitrogen, Carlsbad, CA).

Carbamazepine plasma concentrations at steady-state were determined by high-performance liquid chromatography (HPLC) according to Jankovic et al. (8), using a Chrompack ISOS/GRAS pump (Chrompack, Middelburg, The Netherlands), UV-VIS Chrompack detector (Chrompack) and Spectra Physics 4600 Data Jet integrator (Spectra Physics, San Jose, CA, USA). Samples were prepared by liquid–liquid extraction with diethyl ether. LiCrospher analytical columns (4.6 x 250 mm, 5 µm) with methanol:water:glacial acetic acid (55:44:1) and a 1 ml/min flow rate were used for molecules separation.

Statistical analysis

Genotype data were presented as haplotype and genotype frequencies, and the 95% confidence interval calculations were calculated according to the modified Wald method. Chi-squared tests were used to compare the observed and expected allele frequencies (Hardy- Weinberg equilibrium) as well as the values obtained from previously reported allele frequencies from other populations. The effects of genotype on carbamazepine dosage requirements, plasma concentrations and clearance were examined by Student's t-tests for independent groups. Carbamazepine clearance was estimated based on the assumed 12-h half-life of carbamazepine in children (24). Statistical analyses were performed with Statistica, version 7.1 (StatSoft, Tulsa, OK, USA). P<0.05 was considered statistically significant.

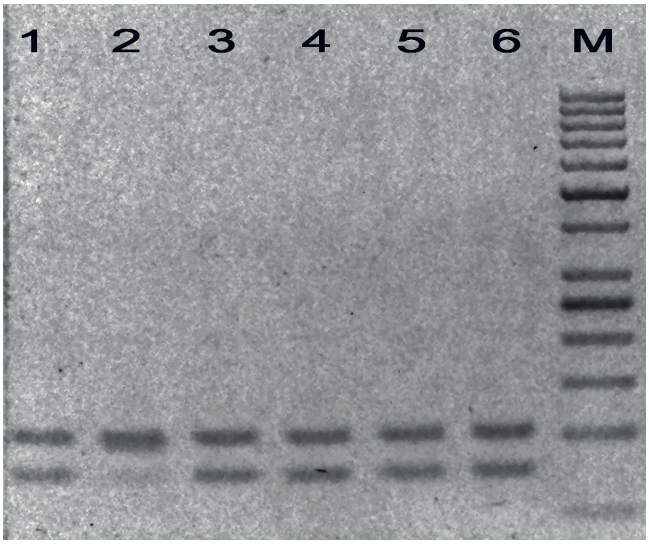

Figure 2. PCR-RFLP analysis of *CYP3A5*3* allele.
Lanes 1, 3-6: 6986G/G genotype; lane 2: 6986A/G genotype; lane M: 50 bp DNA ladder

Table 2. Nucleotide change, haplotype and genotype frequencies of *CYP3A5* in Serbian paediatric epileptic patients on carbamazepine treatment

		Observed frequency	95% Confidence interval
Nucleotide change			
	27289C>A	0.000 (0/80)	0.000, 0.056
	6986A>G	0.975 (78/80)	0.907, 0.998
Haplotype			
	*CYP3A5*1A*	0.025 (2/80)	0.000, 0.093
	*CYP3A5*2*	0.000 (0/80)	0.000, 0.056
	*CYP3A5*3*	0.975 (78/80)	0.907. 0.998
Genotype			
	*CYP3A5*1A/*3*	0.050 (2/40)	0.893, 0.989
	*CYP3A5*3/*3*	0.950 (38/40)	0.893, 0.989

Table 3. *CYP3A5*2* and *CYP3A5*3* distribution in Caucasian populations

Population	CYP3A5 *2 allele frequency	CYP3A5 *3 allele frequency	Reference
Serbian	0.00 (0/80)	0.98 (78/80)	present study
Bosnian		0.93 (259/278)	(48)
Macedonian		0.92 (320/348)	(49)
Greek		0.94 (532/566)	(35)
Italian		0.93 (93/100)	(50)
Polish		0.94 (376/400)	(51)
British		0.94 (188/200)	(23)
Dutch	0.01 (10/1000)	0.92 (920/1000)	(22)
	0.01 (2/200)	0.94 (188/200)	(52)
Finish		0.92 (826/898)	(36)
Russian		0.94 (368/392)	(53)
Australian		0.95 (110/116)	(54)

RESULTS

Genotyping for *CYP3A5*2* and *CYP3A5*3* was performed on 40 Serbian paediatric epileptic patients on carbamazepine treatment, and the nucleotide change, haplotype and genotype distributions are presented in Table 2. All *CYP3A5* genotype frequencies were in accordance with Hardy-Weinberg equilibrium ($\chi 2<0.026$, p=0.05). *CYP3A5*2* was not found. All valproate users were homozygous carriers of *CYP3A5* wild type alleles 27289C and 6986A.

Comparisons with the *CYP3A5* variant allele frequencies observed earlier in other Caucasian populations (Table 3) revealed no significant differences between our results and previously published data in terms of both *CYP3A5*3* ($\chi^2<3.20$, df=1, p>0.07) and *CYP3A5*2* ($\chi 2=0.81$, df=1, p=0.37). Similarly, no differences in *CYP3A5*3* allele frequencies were observed among epileptic patients in comparison to healthy volunteers within different ethnic populations (Table 4, $\chi 2<3.00$, df=1, p>0.08).

CYP3A5 genotype groups did not differ significantly in terms of carbamazepine dosage requirements (p=0.26), dose-normalized plasma carbamazepine concentrations (p=0.47) or carbamazepine clearance (p=0.79). However, there was a tendency towards observed lower dosage requirements (mean ± SD: 15.06 ± 4.45 mg/kg vs. 18.74 ± 5.55 mg/kg), higher plasma concentrations (mean ± SD: 0.45 ± 0.13 mg/kg vs. 0.38 ± 0.03 mg/kg) and lower clearance (mean ± SD: 0.14 ± 0.05 mg/kg vs. 0.15 ± 0.01 mg/kg) in homozygous carriers of *CYP3A5*3/*3* compared to heterozygous *CYP3A5*1A/*3*.

DISCUSSION

In the present study, we investigated the distribution of *CYP3A5*2* and *CYP3A5*3* variants as well as their effects on carbamazepine metabolism in Serbian paediatric epileptic patients. To the best of our knowledge, this is the first study of *CYP3A5* genetic polymorphism in relation to carbamazepine in Serbs. Our results indicate similar frequencies of *CYP3A5* alleles in our Serbian population to those found in other Caucasians and in epileptic patients compared to healthy populations of the same ethnic background. Based on our findings, the proposed effects of *CYP3A5*3* on carbamazepine metabolism in Serbian epileptic patients seem to be possible, but due to the extremely high frequency of *CYP3A5*3* and the small sample size, the effects could not be confirmed.

CYP3A5 is located on chromosome 7q21-q22.1, in a 231-kb cluster with other five members of the CYP3A subfamily:

Table 4. *CYP3A5*3* allelic distribution in epileptic patients compared to healthy volunteers within different populations

Population	Epileptic patients	Healthy volunteers	Reference
Serbian	0.98 (78/80)		present study
		0.92 (252/274)	(55)
		0.94 (113/120)	(56)
Polish	0.88 (130/148)		(47)
		0.85 (120/142)	(47)
Korean	0.77 (54/70)		(15)
		0.70 (70/100)	(30)
Chinese	0.79 (133/168)		(16)
		0.78 (471/604)	(33)
		0.76 (164/216)	(57)
		0.73 (658/902)	(58)
Japanese	0.73 (210/288)		(17)
		0.76 (284/374)	(34)
African–American	0.36 (22/60)		(18)
		0.30 (53/178)	(31)
		0.27 (80/292)	(32)

three genes (*CYP3A4, CYP3A7* and *CYP3A43*) and two pseudogenes (*CYP3A5P1* and *CYP3A5P2*) (25, 26). The gene is highly polymorphic, with more than 25 alleles (http://www.cypalleles.ki.se/cyp3a5.htm) identified to date. It has been shown that the expression of the enzyme is possible only in carriers of at least one wild type *CYP3A5*1A* allele and that the most of the variant alleles are functionally defective (27, 28). The first genetic variant, *CYP3A5*2*, represents a non-synonymous substitution in exon 11 (27289C>A) that leads to an amino acid change at residue 398 (T398N), causing decreased stability and reduced hepatic content of the CYP3A5 protein (27). *CYP3A5*2* is found to be rare in all populations, ranging from 0% in Asians and Blacks (29, 30) to 1% in Caucasians (22). On the other hand, the most frequent and functionally important *CYP3A5* variation, *CYP3A5*3*, is intronic, and consists of a 6986A>G substitution that generates a cryptic splice site and exon 3B, introducing a stop codon and premature termination of a protein translation (28). *CYP3A5*3* represents the most common cause of CYP3A5 loss of expression, found in approximately 30% of African–Americans (31, 32), 75% Asians (33, 34) and more than 90% of Caucasians (22, 35, 36). In the present study, we did not observe carriers of CYP3A5*2 among Serbs, whereas all of the participants were carriers of at least one *3 allele. The results obtained correspond well to the previously published data for Caucasians.

The CYP3A5 enzyme is primarily extrahepatic (10), suggesting that it might play a role in other biological processes in addition to metabolism (28). It has been observed that its level and activity correlates with the risk of developing several diseases, including hypertension (31), acute lymphoblastic leukaemia (37), chronic myeloid leukaemia (38), or breast cancer (39). Epilepsy has a strong hereditary background that involves mutations in multiple genes (40, 41), thus genetic variability in metabolism might potentially contribute to the aetiology of this disease as well. However, based on our results and previously published data, frequency distributions of the most important CYP3A5 variant do not differ between epileptic and non-epileptic subjects within the same populations. Therefore, it is highly unlikely that *CYP3A5* polymorphism represents a risk factor for epilepsy development.

It is well known that metabolism of many drugs, including carbamazepine, largely depends on CYP3A activity (7, 9). Although CYP3A4 plays the leading role, CYP3A5 could contribute substantially, depending on its expression (11). Yet, previous investigations dealing with the influence of *CYP3A5* genotype on drug disposition seem to be conflicting, reporting significant effects in some (12-14, 42, 43) but not all (44-46) CYP3A substrates. Studies on carbamazepine also yielded contradictory results, most probably due to the different ethnic origins of the participants. Namely, the effects of *CYP3A5* genotype on carbamazepine serum concentrations or half-life were observed in Asians and Blacks (15-18) but not in a Caucasian population (18). Similarly, no association between *CYP3A5* polymorphism and carbamazepine resistance was detected in Caucasians (47). In the present study, comparisons between carriers and non-carriers of the non-functional *CYP3A5*3* allele in terms of carbamazepine-pharmacokinetics parameters did not show significant differences. However, a tendency towards lower dosage requirements, higher plasma concentrations and lower clearance of carbamazepine was observed in subjects having the non-functional *CYP3A5* genotype. The lack of statistical significance could be explained by the extremely high frequency of *CYP3A5*3/*3* carriers (95%) but also by the small sample size, caused by a low number of available subjects that met the inclusion criteria for the study. Additional investigations would be necessary to determine the importance of *CYP3A5* genotyping in Caucasian epileptic patients undergoing carbamazepine treatment.

In conclusion, the frequency distribution of *CYP3A5*2* and *CYP3A5*3* alleles in Serbian epileptic patients corresponds well to previously published data for Caucasians. *CYP3A5* polymorphism does not seem to represent a risk factor for epilepsy development. The proposed effects of *CYP3A5*3* on carbamazepine metabolism, although possible, could not be confirmed.

ACKNOWLEDGEMENTS

The study was financially supported by the Faculty of Medical Sciences, University of Kragujevac, Serbia, JP 07/11, and the Ministry of Science and Technology of the Republic of Serbia, grants No. 175007 and 175056.

REFERENCES

1. Abraham S, Shaju M. Innovations in epilepsy management - an overview. J Pharm Pharm Sci 2013; 16(4):564-76.
2. Atlas: Epilepsy Care in the World. Geneva, Switzerland: World Health Organization; 2005. 91p.
3. Magiorkinis E, Diamantis A, Sidiropoulou K, Panteliadis C. Highights in the history of epilepsy: the last 200 years. Epilepsy Res Treat 2014; 2014:582039.
4. Simonato M, French JA, Galanopoulou AS, O'Brien TJ. Issues for new antiepilepsy drug development. Curr Opin Neurol 2013; 26(2):195-200.
5. Laxer KD, Trinka E, Hirsch LJ, et al. The consequences of refractory epilepsy and its treatment. Epilepsy Behav 2014; 37C:59-70.
6. Löscher W, Klotz U, Zimprich F, Schmidt D. The clinical impact of pharmacogenetics on the treatment of epilepsy. Epilepsia 2009; 50(1):1-23.
7. Thorn CF, Leckband SG, Kelsoe J, et al. PharmGKB summary: carbamazepine pathway. Pharmacogenet Genomics 2011; 21(12):906-10.
8. Jankovic SM, Jovanovic D, Milovanovic JR. Pharmacokinetic modeling of carbamazepine based on clinical data from Serbian epileptic patients. Methods Find Exp Clin Pharmacol 2008; 30(9):707-13.

9. Kerr BM, Thummel KE, Wurden CJ, et al. Human liver carbamazepine metabolism. Role of CYP3A4 and CYP2C8 in 10,11-epoxide formation. Biochem Pharmacol 1994; 47(11):1969-79.
10. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacology & Therapeutics 2013; 138:103-41.
11. Lee SJ, Usmani KA, Chanas B, et al. Genetic findings and functional studies of human CYP3A5 single nucleotide polymorphisms in different ethnic groups. Pharmacogenetics 2003; 13:461–472.
12. Hesselink DA, van Schaik RHN, van der Heiden IP, et al. Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. Clin Pharmacol Ther 2003; 74:245-54.
13. Jin Y, Wang YH, Miao J, et al. Cytochrome P450 3A5 genotype is associated with verapamil response in healthy subjects. Clin Pharmacol Ther 2007; 82:579–85.
14. Josephson F, Allqvist A, Janabi M, et al. CYP3A5 genotype has an impact on the metabolism of the HIV protease inhibitor saquinavir. Clin Pharmacol Ther 2007; 81:708-12.
15. Park PW, Seo YH, Ahn JY, et al. Effect of CYP3A5*3 genotype on serum carbamazepine concentrations at steady-state in Korean epileptic patients. J Clin Pharm Ther 2009; 34(5):569-574.
16. Meng H, Ren J, Lv Y, et al. Association study of CYP3A5 genetic polymorphism with serum concentrations of carbamazepine in Chinese epilepsy patients. Neurology Asia 2011; 16(1):39-45.
17. Seo T, Nakada N, Ueda N, et al. Effect of CYP3A5*3 on carbamazepine pharmacokinetics in Japanese patients with epilepsy. Clin Pharmacol Ther 2006; 79(5):509-10.
18. Puranik YG, Birnbaum AK, Marino SE, et al. Association of carbamazepine major metabolism and transport pathway gene polymorphisms and pharmacokinetics in patients with epilepsy. Pharmacogenomics 2013; 14(1):35-45.
19. Chen ML. Ethnic or racial differences revisited: impact of dosage regimen and dosage form on pharmacokinetics and pharmacodynamics. Clin Pharmacokinet 2006; 45(10):957-64.
20. Yasuda SU, Zhang L, Huang SM. The role of ethnicity in variability in response to drugs: focus on clinical pharmacology studies. Clin Pharmacol Ther 2008; 84(3):417- 23.
21. Yokoi T. Essentials for starting a pediatric clinical study (1): Pharmacokinetics in children. J. Toxicol. Sci. 2009; 34(II):SP307-SP312.
22. van Schaik RH, van der Heiden IP, van den Anker JN, Lindemans J. CYP3A5 variant allele frequencies in Dutch Caucasians. Clin Chem 2002; 48(10):1668-71.
23. King BP, Leathart JB, Mutch E, et al. CYP3A5 phenotype-genotype correlations in a British population. Br J Clin Pharmacol 2003; 55(6):625-9.
24. Sweetman S, ed. Martindale: The complete drug reference 36. Pharmaceutical Press; 2009.
25. Spurr NK, Gough AC, Stevenson K, Wolf CR. The human cytochrome P450 CYP3A locus: assignment to chromosome 7g22-qter. Hum Genet 1989; 81(2):171-4.
26. Chen X, Wang HW, Zhou G, et al. Molecular population genetics of human CYP3A locus: signatures of positive selection and implications for evolutionary environmental medicine. Environmental Health Perspectives 2009; 117(10):1541-8.
27. Xie HG, Wood AJJ, Kim RB, et al. Genetic variability in CYP3A5 and its possible consequences. Pharmacogenomics 2004; 5(3):243–272.
28. Kuehl P, Zhang J, Lin Y, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet 2001; 27:383-391.
29. Hiratsuka M, Takekuma Y, Endo N, et al. Allele and genotype frequencies of CYP2B6 and CYP3A5 in the Japanese population. Eur J Clin Pharmacol 2002; 58:417-421.
30. Hustert E, Haberl M, Burk O, et al. The genetic determinants of the CYP3A5 polymorphism. Pharmacogenetics 2001; 11:773-779.
31. Givens RC, Lin YS, Dowling AL, et al. CYP3A5 genotype predicts renal CYP3A activity and blood pressure in healthy adults. J Appl Physiol (1985) 2003; 95(3):1297-300.
32. Bhatnagar V, Garcia EP, O'Connor DT, et al. CYP3A4 and CYP3A5 polymorphisms and blood pressure response to amlodipine among African-American men and women with early hypertensive renal disease. Am J Nephrol 2010; 31(2):95-103.
33. Hu YF, He J, Chen GL, et al. CYP3A5*3 and CYP3A4*18 single nucleotide polymorphisms in a Chinese population. Clin Chim Acta 2005; 353:187–192.
34. Saeki M, Saito Y, Nakamura T, et al. Single nucleotide polymorphisms and haplotype frequencies of CYP3A5 in a Japanese population. Hum Mutat 2003; 618:1-7.
35. Arvanitidis K, Ragia G, Iordanidou M, et al. Genetic polymorphisms of drug-metabolizing enzymes CYP2D6, CYP2C9, CYP2C19 and CYP3A5 in the Greek population. Fundam Clin Pharmacol 2007; 21(4):419-26.
36. Hilli J, Rane A, Lundgren S, et al. Genetic polymorphism of cytochrome P450s and P-glycoprotein in the Finnish population. Fundamental & Clinical Pharmacology 2007; 21:379–86.
37. Borst L, Wallerek S, Dalhoff K, et al. The impact of CYP3A5*3 on risk and prognosis in childhood acute lymphoblastic leukemia. Eur J Haematol 2011; 86(477–83).
38. Sailaja K, Rao DN, Rao DR, Vishnupriya S. Analysis of CYP3A5*3 and CYP3A5*6 gene polymorphisms in Indian chronic myeloid leukemia patients. Asian Pac J Cancer Prev 2010; 11(781-4).
39. Shimada N, Iwasaki M, Kasuga Y, et al. Genetic polymorphisms in estrogen metabolism and breast cancer risk in

case-control studies in Japanese, Japanese Brazilians and non-Japanese Brazilians. J Hum Genet 2009; 54(209-15).
40. Mefford HC. CNVs in Epilepsy. Curr Genet Med Rep 2014; 2:162-167.
41. Helbig I, Lowenstein DH. Genetics of the epilepsies: where are we and where are we going? Curr Opin Neurol 2013; 26(2):179-85.
42. Kim KA, Park PW, Lee OJ, et al. Effect of CYP3A5*3 genotype on the pharmacokinetics and pharmacodynamics of amlodipine in healthy Korean subjects. Clin Pharmacol Ther 2006; 80:646-56.
43. Kim KA, Park PW, Lee OJ, et al. Effect of polymorphic CYP3A5 genotype on the single-dose simvastatin pharmacokinetics in healthy subjects. J Clin Pharmacol 2007; 47:87-93.
44. Eap CB, Buclin T, Hustert E, et al. Pharmacokinetics of midazolam in CYP3A4- and CYP3A5-genotyped subjects. Eur J Clin Pharmacol 2004; 60:231-6.
45. Fukuda T, Onishi S, Fukuen S, et al. CYP3A5 genotype did not impact on nifedipine disposition in healthy volunteers. Pharmacogenomics J 2004; 4(1):34-9.
46. Yamamoto T, Kubota T, Ozeki T, et al. Effects of the CYP3A5 genetic polymorphism on the pharmacokinetics of diltiazem. Clin Chim Acta 2005; 362(1-2):147-54.
47. Emich-Widera E, Likus W, Kazek B, et al. CYP3A5*3 and C3435T MDR1 polymorphisms in prognostication of drug-resistant epilepsy in children and adolescents. Biomed Res Int 2013; 2013:526837.
48. Semiz S, Dujic T, Ostanek B, et al. Analysis of CYP3A4*1B and CYP3A5*3 polymorphisms in population of Bosnia and Herzegovina. Med Glas (Zenica) 2011; 8:84-9.
49. Jakovski K, Kapedanovska Nestorovska A, Labacevski N, Dimovski AJ. Frequency of the most common CYP3A5 polymorphisms in the healthy population of the Republic of Macedonia. Macedonian pharmaceutical bulletin 2012; 58(1,2):25-30.
50. Turolo S, Tirelli AS, Ferraresso M, et al. Frequencies and roles of CYP3A5, CYP3A4 and ABCB1 single nucleotide polymorphisms in Italian teenagers after kidney transplantation. Pharmacol Rep 2010; 62:1159–69.
51. Adler G, Loniewska B, Parczewski M, et al. Frequency of common CYP3A5 gene variants in healthy Polish newborn infants. Pharmacol Rep 2009; 61:947–51.
52. Bosch TM, Doodeman VD, Smits PHM, et al. Pharmacogenetic screening for polymorphisms in drug-metabolizing enzymes and drug transporters in a Dutch population. Mol Diag Ther 2006; 10(3):175-185.
53. Seredina TA, Goreva OB, Talaban VO, et al. Association of cytochrome P450 genetic polymorphisms with neoadjuvant chemotherapy efficacy in breast cancer patients. BMC Med. Genet 2012; 13:45.
54. Wong M, Balleine RL, Collins M, et al. CYP3A5 genotype and midazolam clearance in Australian patients receiving chemotherapy. Clin Pharmacol Ther 2004; 75(6):529-38.
55. Djordjevic N, Jankovic S, Bertilsson L, Aklillu E. CYP3A4 i CYP3A5 genetski polimorfizam kod Srba. Med Cas 2008; 42(1):Suppl 1: 29.
56. Stefanovic N, Cvetkovic T, Velickovic-Radovanovic R, et al. Significance of CYP3A5 gene polymorphism in Serbian renal transplant patients. Acta Medica Medianae 2013; 52(1):33-8.
57. Balram C, Zhou Q, Cheung YB, Lee EJD. CYP3A5*3 and *6 single nucleotide polymorphisms in three distinct Asian populations. Eur J Clin Pharmacol 2003; 59:123–126.
58. Liu CH, Peck K, Huang JD, et al. Screening CYP3A single nucleotide polymorphisms in a Han Chinese population with a genotyping chip. Pharmacogenomics 2005; 6(7):731-47.