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ANALYSING CYP2D6*4 ALLELE FREQUENCY IN PATIENTS WITH SCHIZOPHRENIA

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Cytochrome P450 enzyme superfamily is involved in the metabolism of a range of endogenous and exogenous substrates. The CYP2D6 variant is involved in the metabolism of dozens of drugs such as tricyclic antidepressants, antipsychotics, beta-blockers, anti-arrhythmics, anti-diabetics, anticancer drugs and so on. CYP2D6 enzyme exhibits high polymorphism and the most frequent variant allele CYP2D6*4 is a poor metabolizer (PM). PM causes the reduction of therapeutic response, increase the risk of adverse drug reactions and increase the plasma concentration of both drug and its metabolites above the levels of toxicity. The aim of this study was to analyze CYP2D6*4 allele frequency among schizophrenic patients for further individualisation and rationalisation of therapy. For that purpose we recruited 38 schizophrenic patients and 110 healthy individuals. Allele-specific PCR was used to detect of CYP2D6*4 allele. In 55% of schizophrenic patients we found both wild type allele carriers, in 45% wild type/*4 heterozygous, while *4/*4 homozygous was not identified. A statistically significant difference in the genotype distribution ($p < 0.05$) between schizophrenic patients and healthy individuals was noted. The frequency of allele *4 (37%) is significantly higher in schizophrenics compared to controls, which indicates caution in administration of CYP2D6 substrates. A lower frequency of PMs in schizophrenic patients than in healthy individuals could be explained with CYP2D6 neuroactive substrate metabolism. However, 45% of the schizophrenic patients, who are intermediate metabolizers, carry the higher risk of adverse response to CYP2D6 substrates comparing to wild type. Since none of the analyzed patient was PM, it can be concluded that they received an adequate dose of medication.

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Key words: *schizophrenia, CYP2D6*4, allele, allele specific PCR*

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Introduction

Cytochrome P450 2D6 (CYP2D6) is a member of the cytochrome P450 mixed-function oxidase system. This family is involved in various oxidation/ reduction reactions used in the metabolism of a range of endogenous and exogenous substrates (1). Although the 2D6 isoenzyme is represented by 1.5%, it metabolizes 25% of drugs that are metabolized via cytochrome, which makes up 7-10% of all drugs on the market (2). The CYP2D6 enzyme variant exhibits high polymorphism due to allelic combinations more than 100 different alleles. Most people carry two functional alleles (CYP2D6*1- "wild type" or CYP2D6*2) and are able to metabolize CYP2D6 substrates extensively. In 7-10% of Caucasian Europeans, CYP2D6 enzyme activity is absent due to inheritance of two non-functional alleles (e.g. CYP2D6*3, *4, *5, *6). These individuals are classified as slow (poor) metabolizers (PM). People with one non-functional and one functional allele are considered intermediate metabolizers (IM). The term is also used for persons with one non-functional allele and one allele encoding an enzyme with reduced function, or for persons in whom both alleles encode an enzyme with reduced function (e.g. *10, *17, *41). Ultra-fast metabolizers (UM) have more than two functional copies of the 2D6 gene (mainly CYP2D6*2), exhibit very intense enzymatic activity, are most common among residents of Northeast Africa and Saudi Arabia, while the incidence in Europeans is 1-5% (3-6). Generally, it has been shown that mutations of CYP2D6 are more prevalent in certain countries in the African and Asian continents than in some European countries (2, 7). In a South India, the prevalence of

CYP2D6 alleles in the descending order was CYP2D6*1, *2, *10, *4, and *5 (8). CYP2D6*4 is the most frequent variant allele in Caucasian Europeans (20%) and in the Middle Eastern countries (9), and it is the leading cause of the slow metabolizer phenotype. Over 75% of slow metabolizers are carriers of this polymorphism (8). The most common inactivating mutation in Caucasian Europeans is the G1934A substitution at the intron extrusion site (CYP2D6*4 allele), which leads to the synthesis of truncated protein and the absence of catalytic activity. G u A transition at the intron 3/exon 4 boundary of the CYP2D6 gene causes improper excretion of introns on the mRNA and results in mutation of the transcription frame shift and premature termination. The G to A transition has been identified as a primary defect of the CYP2D6 locus and is present in 80-90% of mutant alleles in slow metabolizers (10).

Due to the decrease in CYP2D6 activity, the metabolism of drugs, that are primarily metabolized via CYP2D6 decreases, which increases the plasma concentrations of these drugs and increases the risk of dose-dependent side effects. Fast metabolizers (*1/*1 homozygotes) are thought to carry a lower risk of developing drug intolerance, dose-dependent adverse reactions and drug toxicity symptoms compared to intermediate metabolizers (1*/4* heterozygotes). Slow metabolizers require lower maintenance doses compared to fast metabolizers. There are insufficient data on initial doses, time to target plasma drug concentrations, first dose effects, and response to change in therapy, most likely due to the large number of different pharmacological groups of drugs metabolized by CYP2D6 (6, 8).

Schizophrenia is the most devastating chronic psychiatric disorder expressing in many different clinical forms. Clinically, patients may show positive symptoms (delusions, hallucinations, agitation or catatonia), negative symptoms (social withdrawal with lack of affective responses, apathy, anhedonia and impaired thought and speech content) or may exhibit both types of symptoms simultaneously. For the treatment of such complex symptomatology, doctors have at their disposal a range of antipsychotic drugs that are equally effective in the treatment of psychotic symptoms and differ in the type of their side effects and sedative effect. First-generation antipsychotics (chlorpromazine) cause a number of side effects of anticholinergic and extrapyramidal origin. Therefore, the first line in the treatment of schizophrenia consists of second-generation antipsychotics (olanzapine, risperidone and quetiapine), which cause fewer side effects. Clozapine is reserved for the treatment of resistant schizophrenia and requires careful monitoring and control, because in addition to the characteristic side effects, it also causes severe agranulocytosis (11). The concentration of antipsychotics in plasma is particularly affected by the metabolism of CYP2D6, which is of great importance due to the narrow therapeutic index of these drugs. Side effects (perphenazine, haloperidol and thioridazine) such as excessive sedation and Parkinson's side effects are associated with changes in CYP2D6 metabolism, while the effect on tardive dyskinesia, acute dystonia of extrapyramidal symptoms and akathisia has not been established (7, 8, 12). As the 2D6 isoenzyme is the only non-inducible among CYP450 enzymes and is not regulated by any known environmental factors, genetic variations contribute greatly to inter-individual differences in enzyme activity (8).

Materials and methods

Patients

A total of 148 subjects, 38 patients with schizophrenia and 110 healthy subjects were included in the study. Patients were recruited in the Specialized Hospital for Psychiatric Diseases "Gornja Toponica" in Niš, with a diagnosis of chronic schizophrenia, who were on appropriate pharmacological therapy. Psychiatric symptoms were monitored using The Positive and Negative Syndrome Scale (PANSS).

CYP2D6*4 genotyping

Genetic testing of CYP2D6 polymorphism was performed in the Laboratory for Functional Genomics and Proteomics of the Scientific Research Center for Biomedicine of the Medical Faculty in Niš. DNA was isolated from whole blood samples supplemented with EDTA, standard Na-dodecyl sulfate lysis procedures, proteinase K digestion, phenol/chloroform extraction and ethanol precipitation, and a commercial isolation kit (Fermentas, Terhmo Fischer Scientific Inc). Detection of CYP2D6*4 was performed by amplification of the desired gene segment, allele specific polymerase chain reaction (ASPCR), using 4 primers, marked as:

1: 5'-TCCCAGCTGGAATCCGGTGTCG-3'
2: 5'-GGAGCTCGCCCTGCAGAGACTCCT-3'
7: 5'-CGAAAGGGGCGTCC-3'
11: 5'-TCTCCCACCCCCAA-3'

The 25 μL PCR reaction mixture contained: 12.5 μL Kappa Mix (Fermentas), 10.2 μL ultrapure water, 0.5 μL primer 1, 0.5 μL primer 2, 0.5 μL primer 7, 0.5 μL primer 11 and 0.3 μL DNA (50 ng/mL). The amplification program of the CYP2D6 gene allele 4 is shown in Table 1.

The obtained PCR products were further analyzed by horizontal electrophoresis, on a 1.5% agarose gel with TBE buffer and ethidium bromide as amplifier detection agent, for 1.25 h, and detection was performed on a transilluminator under a UV lamp. DNA was subsequently reanalyzed to confirm the results of the CYP2D6*4 assay.

Statistical analysis. Statistical processing of the results was performed using the SPSS statistical program 15.0, χ^2 test and Fisher's accuracy test. $P < 0.05$ was considered statistically significant.

Results

By allele-specific PCR, CYP2D6*4 allele was detected as slow metabolizing form of CYP enzyme. A 750 bp fragment was amplified first (primers 1 and 2), followed by ASA PCR to give 560 bp(wt) and 217 bp(*4) fragments. The distribution of genotypes in patients with schizophrenia and non-schizophrenics is shown in Table 2.

The distribution of genotypes in patients with schizophrenia is significantly different compared to healthy subjects. A heterozygote wild-type/*4 was identified in 45% of patients with schizophrenia, as opposed to 22% in controls, while slow metabolizing homozygotes were not observed and significantly lower ($p < 0.05$) compared to the control group. The incidence of CYP2D6*4 alleles was significantly higher in patients with schizophrenia (Table 3) compared to the reference group of control subjects ($p < 0.0001$).

Table 1. CYP2D6 amplification program

Program			
Order of			
1.		Temperature	Time
		95 °C	2 minutes
2.	**First set**		
	Number of cycles	12	
		Temperature	Time
		95 °C	15 seconds
		63 °C	30 seconds
		72 °C	45 seconds
3.	**Second set**		
	Number of cycles	24	
		Temperature	Time
		95 °C	15 seconds
		53 °C	30 seconds
		72 °C	45 seconds
4.	**Final elongation**		
		Temperature	Time
		72 °C	7 minutes

Table 2. Genotype distribution in the CYP2D6 gene in patients with schizophrenia

Genotype	Patients with schizophrenia	Control group
wt/wt	55%	75%
wt/*4	45% ***	22%
*4/*4	-	3%*

* $p < 0.05$; *** $p < 0.001$

Table 3. Frequency of CYP2D6*w or 4 alleles in patients with schizophrenia

Allele	Patients with schizophrenia	Control group
Wild-type	63%	86%
CYP2D6*4	37% ***	14%

*** $p < 0.0001$

Discussion

In this study, a significant difference in genotype distribution was noted, as well as a significantly higher frequency of alleles of *4 of CYP2D6 gene in patients with schizophrenia compared to healthy individuals. There is also a significantly lower prevalence of slow metabolizing homozygotes (*4/*4) in schizophrenic patients than in healthy volunteers. This finding is consistent with the results of other similar studies and could be explained by the involvement of CYP2D6 in the metabolism of endogenous neuroactive substrates (13, 14). It also supports the hypothesis of a potential role of CYP2D6 in the vulnerability to schizophrenia. In the previously published study (15), a significant clinical improvement was found in patients with schizophrenia with the CYP2D6 poor metabolizer phenotype compared with the treatment out comes in extensive metabolizers. However, Kakihara et al. (16) did not identify any significant association between CYP2D6 polymorphisms and clinical recovery. This finding was supported with another study involving female patients with schizophrenia that found clinical improvement following risperidone treatment, but was not associated with CYP2D6 genotype (17). Recently, Lu et al. (18), in a well-designed study on the effect of CYP2D6 polymorphism on the concentration and therapeutic effects of risperidone, have shown that the CYP2D6 genotype exerts a slight effect on improvement of clinical symptoms but has a significant effect on risperidone plasma concentrations. This result suggests that in patients with schizophrenia treated with risperidone CYP2D6 genotype might influence adverse drug reactions. There is evidence that children with CYP2D6 poor or intermediate metabolizer phenotypes are at a greater risk for risperidone adverse effect (19). It was also shown that the plasma concentration of risperidone was significantly different depending on homo - or hetrozygosity of CYP2D6*10 mutations confirming the finding of previous studies showing that homozygous mutations CYP2D6*10 had higher plasma concentrations of risperidone than single-allele carriers (20, 21). Lu et al. (18) also showed that C100T and G4181C polymorphisms were associated with differences in plasma concentration of risperidone and the ratio of risperidone to 9-hydroxyrisperidone suggesting that even single-nucleotide mutations are sufficient to affect the activity of enzymes. It is obvious that plasma concentration varies according to allelic variants. Importantly, this may contribute to the clinical treatment response and may provide new insight for individualized drug treatment.

In contrast to the results obtained on the frequency of slow metabolizers, we found a significantly higher prevalence of CYP2D6*4 alleles and intermediate metabolizers in patients with schizophrenia which is consistent with the findings of Llerena et al. (13). Very recently (22), it has been shown that physiologically based pharmacokinetic model for aripiprazole and dehydro-aripiprazole indicates a dose reduction for CYP2D6 poor metabolizers to achieve steady-state plasma concentrations and suggests a maximum daily dose of 10 mg for patients with schizophrenia, while IMs and UMs do not need a dose adjustment. Further, dose adjustment to the CYP2D6 genotype or phenotype according to the guidelines is not applicable for patients already using antipsychotics (23). That the polymorphism of CYP2D6*10 affects the steady state plasma concentration of risperidone is also confirmed in Indian patients with schizophrenia (24). By examining the possible association between CYP2D6 genotype, hippocampal white matter integrity, and therapeutic responseto antipsychotic drugs in Korean patients with schizophrenia Shin et al. (25) showed that CYP2D6-dependent hippocampal white matter alterations could be an endotype for schizophrenia that accounts for individual differences in clinical features and treatment responses.

Conclusion

Taken together, our results, although we did not identify poor metabolizers, suggest CYP2D6 genotyping combined with drug concentration monitoring in order to personalize the drug dose to achieve efficacy and avoid adverse effects in patients with schizophrenia.

References

1. Lamba V, Lamba JK, Dilawari JB, Kohli KK. Genetic polymorphism of CYP2D6 in North Indian subjects. Eur J Clin Pharmacol 1998;54:787-91. [CrossRef] [PubMed]
2. Fleeman N, Dundar Y, Dickson R, Jorgensen A, Pushpakom S, McLeod C et al. Cytochrome P450 testing for prescribing antipsychotics in adults with schizophrenia: systematic review and meta-analyses. Pharmacogenomics J 2011;11:1-14. [CrossRef] [PubMed]
3. Ma MK, Woo MH, Mcleod HL. Genetic basis of drug metabolism. Am J Health Syst Pharm 2002;59(21): 2061-9. [CrossRef] [PubMed]
4. Sachse C, Brockmoller J, Bauer S, Roots I. Cytochrome P450 2D6 variants in a Caucasian population: Allele frequencies and phenotypic consequences. Am J Hum Genet 1997;60:284-95. [PubMed]
5. Acuña M, Pinto E, Olivares P, Ríos C. Genetic variants of cytochrome *CYP2D6* in two mixed chilean populations. Hum Hered 2016;82:16-20. [CrossRef] [PubMed]
6. Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, Leeder JS. The CYP2D6 activity score: Translating genotype information into a qualitative measure of phenotype. Clinl Pharmacol Therapeutics 2008;83(2):234-42. [CrossRef] [PubMed]
7. Ingelman- Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. The Pharmacogenomics J 2005:15:6-13. [CrossRef] [PubMed]
8. Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. Influence of cytochrome P450 polymorphisms on drug therapies: Pharmacogenetic, pharmacoepigenetic and clinical aspects. Pharmacol Ther 2007;116:496-526. [CrossRef] [PubMed]
9. Khalaj Z, Baratieh Z, Nikpour P, Khanahmad H, Mokarian F, Rasoul Salehi R et al. Distribution of CYP2D6 polymorphism in the Middle Eastern region. J Res Med Sci 2019;24:61. [CrossRef] [PubMed]
10. Surekha D, Sailaja K, Nageswara RD, Padma T, Raghunadharao D, Vishnupriya S. CYP2D6*4 polymorphisms and breast cancer risk. Biol Med 2010; 2(4):49-55.
11. Branford D. Schizophrenia. In: Clinical pharmacy and therapeutics, 5th edition. Walker R, Whittlesea C eds. Elsevier,2012,479-88.
12. Urichuk L, Prior TI, Dursun S, Baker G. Metabolism of atypical antipsychotics: involvement of cytochrome p450 enzymes and relevance for drug-drug interactions. Curr Drug Metab 2008:9(5):410-8. [CrossRef] [PubMed]
13. Llerena A, Naranjo ME, Rodrigues-Soares F, Penas-Lledo EM, Farina H, Tarazona-Santos E. Interethnic variability of CYP2D6 alleles and of predicted metabolic phenotypes across world populations. Expert Opin Drug Metab Toxicol 2014;10:1569-83. [CrossRef] [PubMed]
14. Ozdemir V, Gunes A, Dahl ML, Scordo GM, Williams-Jones B, Someya T. Could endogenous substrates of drug-metabolizing enzymes influence constitutive physiology and drug target responsiveness? Pharmacogenomics 2006;7(8):1199-210. [CrossRef] [PubMed]
15. Almoguera B, Riveiro-Alvarez R, Lopez-Castroman J, Dorado P, VaqueroLorenzo C, Fernandez-Piqueras J et al. CYP2D6 poor metabolizer status might be associated with better response to risperidone treatment. Pharmacogenet Genomics 2013;23(11):627-30. [CrossRef] [PubMed]
16. Kakihara S, Yoshimura R, Shinkai K, Matsumoto C, Goto M, Kaji K et al. Prediction of response to risperidone treatment with respect to plasma concentrations of risperidone, catecholamine metabolites, and polymorphism of cytochrome P450 2D6. Int Clin Psychopharmacol 2005;20:71-8. [CrossRef] [PubMed]
17. Jovanović N, Božina N, Lovrić M, Medved V, Jakovljević M, Peleš AM. The role of CYP2D6 and ABCB1 pharmacogenetics in drug-naïve patients with first-episode schizophrenia treated with risperidone. Eur J Clin Pharmacol 2010;66(11):1109-17. [CrossRef] [PubMed]
18. Lu J, Yang Y, Lu J, Wang Z, He Y, Yan Y, et al. Effect of CYP2D6 polymorphisms on plasma concentration and therapeutic effect of risperidone. BMC Psychiatry 2021;21:70. [CrossRef] [PubMed]
19. Oshikoya KA, Neely KM, Carroll RJ, Aka IT, Maxwell-Horn AC, Roden DM et al. CYP2D6 genotype and adverse events to risperidone in children and adolescents. Pediatr Res 2019;85(5):602-6. [CrossRef] [PubMed]
20. Suzuki Y, Fukui N, Tsuneyama N, Watanabe J, Ono S, Sugai T et al. Effect of the cytochrome P450 2D6*10 allele on risperidone metabolism in Japanese psychiatric patients. Hum Psychopharmacol 2012;27(1):43-6. [CrossRef] [PubMed]
21. Mihara K, Kondo T, Yasui-Furukori N, Suzuki A, Ishida M, Ono S et al. Effects of various CYP2D6 genotypes on the steady-state plasma concentrations of risperidone and its active metabolite, 9-hydroxyrisperidone, in Japanese patients with schizophrenia. Ther Drug Monit 2003;25:287-93. [CrossRef] [PubMed]
22. Kneller LA, Zubiaur P, Koller D, Abad-Santos F, Hempel G. Infuence of CYP2D6 phenotypes on the pharmacokinetics of aripiprazole and dehydroaripiprazole using a physiologically based pharmacokinetic approach. Clin Pharmacokinetics 2021;60:1569-82. [CrossRef] [PubMed]
23. Koopmans AB, Vinkers DJ, Poulina IT, Gelan PJA, van Schaik RHN, Hoek W, et al. No effect of dose adjustment to the CYP2D6 genotype in patients with severe mental illness. Front Psychiatry 2018;9:349. [CrossRef] [PubMed]
24. Chavan BS, Kaur G, Gupta D, Aneja J. A prospective study to evaluate the effect of CYP2D6 polymorphism on plasma level of risperidone and its metabolite in north Indian patients with schizophrenia. Indian J Psychological Med 2018;40(4):335-42. [CrossRef] [PubMed]
25. Shin W, Bang M, Kim A, Cho D-Y, Lee S-H. Influence of cytochrome P450 2D6 polymorphism on hippocampal white matter and treatment response in schizophrenia. NPJ Schizophrenia 2021;7:5. [CrossRef] [PubMed]

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ANALIZA FREKVENCIJE ALELA CYP2D6*4 KOD BOLESNIKA SA SHIZOFRENIJOM

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Superfamilija enzima citohroma P450 uključena je u metabolizam niza endogenih i egzogenih supstrata. Varijanta CYP2D6 uključena je u metabolizam desetina lekova, kao što su triciklični antidepresivi, antipsihotici, beta blokatori, antiaritmici, antidijabetici, lekovi protiv karcinoma i tako dalje. Enzim CYP2D6 pokazuje visok polimorfizam i najčešća varijanta alela CYP2D6*4 je spor metabolizator (SM). SM uzrokuje smanjenje terapijskog odgovora, povećava rizik od neželjenih reakcija na lek i povećava koncentraciju leka i njegovih metabolita u plazmi, iznad nivoa toksičnosti. Cilj ove studije bila je analiza učestalosti alela CIP2D6*4 kod bolesnika sa shizofrenijom, radi dalje individualizacije i racionalizacije terapije. U ispitivanje je uključeno 38 bolesnika sa shizofrenijom i 110 zdravih osoba. PCR specifičan za alel korišćen je za detekciju alela CYP2D6*4. Kod 55% bolesnika sa shizofrenijom pronašli smo oba nosioca alela divljeg tipa, kod 45% heterozigot divlji tip/*4, dok homozigot *4/*4 nije identifikovan. Uočena je statistički značajna razlika u distribuciji genotipa ($p < 0,05$) između shizofrenih bolesnika i zdravih osoba. Učestalost alela *4 (37%) značajno je veća kod obolelih od shzofrenije u poređenju sa ispitanicima kontrolne grupe, što ukazuje na oprez u primeni supstrata CYP2D6. Niža učestalost SM kod shizofrenih bolesnika, nego kod zdravih osoba može se objasniti metabolizmom neuroaktivnog supstrata CYP2D6. Međutim, 45% bolesnika sa shizofrenijom, koji su srednji metabolizatori, nosi veći rizik od neželjenog odgovora na supstrate CYP2D6, u poređenju sa divljim tipom. Kako nijedan od analiziranih bolesnika nije bio SM, može se zaključiti da su bolesnici primali adekvatne doze leka.

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Ključne reči: *shizofrenija, CYP2D6*4, alel, alel specifična PCR*

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