The Genetic Variation of CYP2D6 Gene in the Bosnian Population

Hilada Nefic

ABSTRACT

Introduction: Genetic polymorphism is associated with individual responses to medication and susceptibility to diseases, and it represents the basis for individualized medical treatment and drug genomics studies. Genetic variation at CYP2D6 is high, both among populations and among individuals in the same population. Aim: The aim of the study was to investigate the CYP2D6 gene duplication and the non-synonymous single-nucleotide polymorphisms (SNP) 100C>T in the CYP2D6 gene in members of the Bosnian population. Material and Methods: The blood samples were collected from 151 unrelated and healthy donors from Bosnian populations consisted of 94 females and 57 males. Duplex long-range PCR was used to determine whether individuals carrying CYP2D6 gene duplication. The resulting PCR product of 5.1 kb, containing all nine CYP2D6 exons, was used as template for detection of the CYP2D6 gene duplication and the non-synonymous single-nucleotide polymorphisms (SNP) 100C>T allele by nested PCR. Results: The CYP2D6 gene duplication frequency found in the Bosnian population (2.73%) was related to the frequencies of this allele in other Caucasians. The gene duplication is the result of inheritance of more than two copies of the fully functional CYP2D6 alleles. In contrast, the frequency of the CYP2D6 100C>T variant, with possibly damaging function, in the Bosnian population (15.56%) was significantly higher when compared with the other Caucasians but significantly lower when compared with Asians. Conclusion: CYP2D6 metabolizes many commonly prescribed drugs. Variations in the gene encoding this enzyme have been associated with individual differences in drug metabolism rates. The individuals with multiple CYP2D6 gene copies metabolize drugs more rapidly and therapeutic plasma levels will not be achieved at ordinary drug dosages. The non-synonymous coding SNP (100C>T) were predicted to have damaging effects on the protein function. Keywords: CYP2D6 gene, duplication, 100C>T variant.

1. INTRODUCTION

The polymorphism associated with mutations in the CYP2D6 gene represents the most frequently studied example in the human P450 complement. The gene “cytochrome P450, family 2, subfamily D, polypeptide 6” (CYP2D6) encodes a member of the cytochrome P450 superfamily of enzymes (debrisoquine 4-hydroxy-lase). The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. These enzymes are responsible for elimination of numerous endogenous substrates and a diverse array of drugs. In mammals, most xenobiotics are metabolized by CYP monooxygenases. Among the drug-metabolizing CYPs, CYP2D6 is the only non-inducible enzyme, which results in a large contribution of genetic variation to the inter-individual variation in enzyme activity. The CYP2D6 protein localizes to the endoplasmic reticulum and it is known to metabolize as many as 20% of commonly prescribed drugs (1) including debrisoquine, an adrenergic blocking drug; antiarrythmic drugs; and an anti-depressant.

The CYP2D6 gene encoding this protein is mapped on chromosome 22q13.1 (OMIM 124030), at the 3’ end of the CYP2D cluster, downstream of highly homologous inactive pseudogenes CYP2D7P and CYP2D8P (2, 3) on the minus chromosomal strand. The CYP2D6 gene consists of nine exons with an open reading frame of 1,491 base pairs coding for 497 amino acids. As a consequence of its highly polymorphic character, more than 100 CYP2D6 allele variants have been described today by the Human Cytochrome P450 Nomenclature Committee (http://www.cypalleles.ki.se/). Besides single-nucleotide polymorphisms (SNPs) or small insertions and deletions, this gene locus also undergoes recombination events creating alleles with large deletions, duplications, conversions, copy number variations...
(CNVs), including deletion and duplication/multiplications of whole gene.

In addition to the wild-type gene (CYP2D6*1), allelic variants of CYP2D6 are associated with deficient, normal, reduced, or increased enzyme activity detected in Caucasians. Accordingly, the conventional phenotypic classification system defines predicted CYP2D6 activity as poor metabolizer (PM), extensive metabolizer (EM), intermediate metabolizer (IM) and ultra-rapid metabolizer (UM) phenotypes. UM and PM are those most at risk for treatment failure or dose-dependent drug toxicity. Genetic variation at CYP2D6 is high, both among populations and among individuals of the same population. This diversity of alleles and the incidence of phenotypes vary among ethnic groups.

Despite the importance for clinical pharmacogenomics and precision medicine, no current “gold standard” method exists for clinical determination of CYP2D6. Current genotyping techniques targeting the CYP2D6 variant alleles have facilitated phenotype prediction from the genotype information without the need for the administration of CYP2D6 probe drugs (4).

2. AIM

Single nucleotide polymorphisms are the most common type of genetic variation among people. Non-synonymous SNPs (nsSNPs) are SNPs located in coding regions and resulting in amino acid variation in protein product of genes. The aims of the study were to investigate a region of chromosome 22 containing part of the first exon of CYP2D6 gene including 100C>T variant with nested PCR and to determine subjects carrying duplication/multiplications of whole gene in the Bosnian population using long-range PCR. After that, effects of examined SNP on protein structure and function was predicted.

3. MATERIAL AND METHODS

3.1. Subjects

The blood samples were collected from 151 unrelated and healthy donors from Bosnian populations consisted of 94 females and 57 males between 19 and 74 years of age. The mean age of subjects was 34.97±13.91 years; the mean age of females and males were 35.47±13.84 and 34.16±14.88 years, respectively. Blood samples (3 ml) were taken and collected into tubes with EDTA between age. The mean age of subjects was 34.97±14.21 years; the mean ages of the female and males were 35.47±13.84 and 34.16±14.88 years, respectively. Blood samples (3 ml) were taken and collected into tubes with EDTA between 2011 and 2015. Fully individuals who participated in this study belonged to Caucasians from different regions of Bosnia and Herzegovina. All the subjects included in this study were approved by the FederalMinistry of Education and Science (05-39-3627-1/14) and the Ethics Committee of the Faculty of Science on University of Sarajevo (01/01-2473/2-2015).

3.2. Detection of CYP2D6 gene duplication

Genomic DNA was extracted from whole blood according to a previously described method by Miller et al. (5) with some modifications. The 5.1 kb fragment that covers the complete CYP2D6 gene and 3.5 kb fragment, if the multiplication allele is present, were amplified by duplex long-range PCR (XL-PCR) using JumpStart™ AccuTaq™ LA DNA Polymerase Mix (AccuTaq LA DNA Polymerase and JumpStart Taq antibody; Sigma-Aldrich, Saint Louis, Missouri, USA) and primers 5’- GTTATCCCAGAAGGTTCCTTG AGGTTCCTA-3’ (DPKup, forward) and 5’- GCCGACTGAGCCCTG- GGAGGTA GTGA-3’ (DPKlow, reverse) for complete gene and primers 5’- CCTGGGAAGGCCCATG- GAAG-3’(2D6dupl-F, forward) and 5’- CAGTTACGG- CAGTGGTCAGCT-3’ (2D6dupl-R, reverse) for a fragment spanning the potential crossover sites. A forward primer DPKup is specific for the 5’UTR of the CYP2D6 gene and a reverse primer DPKlow for the downstream of the CYP2D6 gene while the forward primer 2D6dupl-F is specific for CYP2D6 3’ flanking sequences and the reverse 2D6dupl-R for a CYP2D7 sequence (REP7).

PCR amplifications were performed on a thermal cycler (Eppendorf Mastercycler gradient, Hamburg, Germany) in 0.2-mL thin-walled tubes using the following PCR reaction mixture: 25 μl contained 50–100 ng of template DNA (genomic DNA), 1x PCR buffer (10X Buffer for AccuTaq LA DNA Polymerase containing 25 mM MgCl) (Sigma-Aldrich, USA), 0.5 mM of each deoxynucleotide triphosphate (Deoxynucleotide Mix, 10mM Solution: 10 mM dATP, 10 mM dCTP, 10 mM dGTP, 10 mM TTP; Sigma, Saint Louis, Missouri, USA), 0.5 μM of each primer (Sigma-Aldrich, USA) and 2.5 U of JumpStart AccuTaq LA DNA Polymerase Mix. Thermal cycling conditions were as follows: initial denaturation step for 1 min at 96 °C, followed by 30 cycles each of 15 seconds at 94 °C (denaturation), 30 seconds at 65 °C (annealing), 6.5 min at 68 °C (extension) followed by one final extension of 30 min at 68 °C. Five μl of PCR products were analyzed by 2% agarose gel electrophoresis and the DNA was visualized with ethidium bromide.

3.3. Detection of the CYP2D6 100C>T variant

The resulting PCR product of 5.1 kb, containing all nine CYP2D6 exons, was used as template for detection of the CYP2D6 100C>T allele by nested PCR that increases the specificity of DNA amplification. The region CYP2D6 gene containing part of the first exon and intron was amplified. All PCR amplifications for CYP2D6 gene were carried out in a thermal cycler in a total volume of 25μl containing 0.32 μM each of the forward 5’- CCT-GATGCACCGGCGCAAGCCTG GGCGTGCACAG- TAC -3’ and reverse 5’- CAAACCTGCTTCCCCTTCT- CAGCC -3’ primer (Sigma, USA), 0.2 mM of each dNTP (Deoxynucleotide Mix; Sigma, USA), 1x PCR reaction buffer (10X PCR buffer without MgCl2; Sigma-Aldrich, USA), 1.5 mM MgCl2 (25 mM magnesium chloride in water; Sigma, USA), 1 U Taq Polymerase (Taq DNA Polymerase, from Thermus aquaticus recombinant; Sigma-Aldrich, USA) and DNA template. The forward primer was designed to introduce a restriction site for the endonuclease Scal in mutant allele. The forward primer was situated within exon 1 and encompasses the 100C>T SNP, as well as the rest of coding region of exon 1 and part of intron 1. The cycling protocol consisted of initial denaturation at 94 °C for 1 min, followed by 30 cycles each of 10 seconds at 94 °C, 20 seconds at 65 °C,
20 seconds at 72 °C and a final extension at 72 °C for 7 min. The amplification products of 241 bp were electrophoresed in 3% agarose gels and stained with ethidium bromide.

3.4. Protocol for Digestion of the PCR Products after Amplification

After the amplification, the nested PCR products were digested with the restriction endonuclease ScaI (New England Biolabs, Beverly, USA) (recognition sequence: 5’-AGT/ACT -3’). The digestion reactions contained: 10 µl of PCR product (~ 0.1 – 0.5µg of DNA), 2.5 µl of buffer (10x Digestion Buffer SH, supplied with the enzyme) and 0.5 µl of enzyme (concentration: 10U/µl) and nuclease-free water 12 μl. These components were incubated for 4 hours at 37 °C. After incubation, 3 µl of 5x loading dye buffer (LDB) was added to the digestion reaction. The digestion products were separated on ethidium bromide-stained 3% agarose gels.

3.5. Statistical analysis

The gene duplication and SNP allele frequencies were calculated by a statistical method. Hardy-Weinberg equilibrium (HWE) for CYP2D6 100C>T variant allele in the Bosnian population was conducted by HW calculator. Comparisons of SNP frequencies between Bosnian population and other ethnic populations were done using a chi-square (χ2) test with one degree of freedom, significance level of 0.05 and 95% confidence intervals (95% CI). All statistical analysis was calculated by MedCalc software version 12.6 (Ostend, Belgium).

3.6. Functional prediction

For predicting damaging effects of variant on the function of the cytochrome P450 enzyme, the online tool PolyPhen-2 (Polymorphism Phenotyping v2) was used. PolyPhen-2 is a software tool that predicts the possible impact of amino acid substitutions on the structure and function of human proteins using straightforward physical and evolutionary comparative considerations (6). PolyPhen-2 features involve comparison of a property of the wild-type allele and the corresponding property of the mutant allele.

4. RESULTS

The study of subjects carrying CYP2D6 gene duplication/multiplication in the Bosnian population revealed that 2.73% of the Bosnians carry more than two active CYP2D6 gene copies. The long range PCR is one of method for analyzing copy number variations in the CYP2D6 gene, but this method cannot determine which of two CYP2D6 alleles in a DNA sample carries duplication or multiplication.

The frequency of the CYP2D6 100C>T variant in the Bosnian population was 15.56% (Table 1). After cytosome to thymine substitution at nucleotide 100 in exon 1 of CYP2D6 gene, the proline is substituted for serine at the 34th amino acid position (p.Pro34Ser) of CYP2D6 protein. The change of an amino acid in this position may decrease the activity of the enzyme. All the genotypes were in Hardy-Weinberg Equilibrium (HWE) (χ²=0.165684; P=0.6839).

### Table 1. The frequencies of CYP2D6 100C>T genetic variants in the Bosnian population. Notes: CC: wild-type allele; CT: heterozygous variant allele; TT: homozygous variant allele; HWE: Hardy-Weinberg Equilibrium

| Genotypes | Observed | Expected |
|-----------|----------|----------|
| CC        | 107      | 107.7    |
| CT        | 41       | 39.7     |
| TT        | 3        | 3.7      |

| Alleles | Observed | Expected |
|---------|----------|----------|
| C       | 255      | 84.44    |
| T       | 47       | 15.56    |

Table 2. The CYP2D6 100C-T variant allele frequencies in the Bosnian and other world populations

| Population  | Sample size (n) | T allele (%) | P value | χ² | 95% CI | References |
|-------------|-----------------|--------------|---------|----|--------|------------|
| Bosnian     | 151             | 15.56        |         |    |        |            |
| Austrian    | 93              | 4.3          | P = 0.0127 | 6.215 | 2.94% - 18.725% | (7)         |
| Spanish     | 105             | 1.9          | P = 0.0007 | 11.411 | 6.447% - 20.646% | (8)         |
| Sardinian   | 250             | 5.4          | P = 0.0012 | 10.422 | 3.701% - 17.374% | (9)         |
| Brazilian   | 873             | 2.05         | P < 0.0001 | 57.144 | 8.003% - 20.345% | (10)        |
| African American | 222 | 3.6          | P = 0.0001 | 15.121 | 5.61% - 19.042% | (11)        |
| Ethiopian   | 122             | 8.6          | P = 0.1218 | 2.394 | -1.442% - 14.998% | (12)        |
| UAE         | 151             | 3.3          | P = 0.0006 | 11.890 | 5.406% - 19.399% | (13)        |
| Turkish     | 100             | 14.5         | P = 0.9605 | 0.00245 | -8.96% - 10.28% | (14)        |
| Uygar       | 96              | 15.6         | P = 0.8638 | 0.0294 | -9.426% - 10.38% | (15)        |
| Chinese Han | 400             | 52.53        | P < 0.0001 | 59.843 | 28.53% - 44.304% | (16)        |
| Korean      | 400             | 45.0         | P = 0.0001 | 39.535 | 21.044% - 36.8% | (17)        |
| Japanese    | 1017            | 42.7         | P = 0.0001 | 39.506 | 19.696% - 33.353% | (18)        |
| Thai        | 288             | 44.6         | P = 0.0001 | 35.641 | 20.094% - 37.058% | (19)        |

Table 3. The CYP2D6 100C-T variant allele frequencies in the Bosnian and other world populations Notes: Values in bold are significant (P<0.05) and were calculated using the χ² test; CI: confidence interval.
Furthermore, the CYP2D6 100C>T allelic variant frequencies were compared between the Bosnian population and other world populations from different geographical regions: Austrian, Spanish, Sardinian, Brazilian, African American, Ethiopian, Turkish, Uygur, Chinese Han, Korean, Japanese and Thai population (Table 2). The CYP2D6 100C>T allele frequency in the Bosnian population was similar to frequencies of this allele in Ethiopian (p = 0.1218; 95% CI: -1.442% to 14.998%), Turkish (P = 0.9605; 95% CI: -8.96% to 10.28%) and Uygur (P = 0.8638; 95% CI: -9.426% to 10.38%) populations.

The frequency of 100C>T SNP in the Bosnian population was significantly higher than those in other Caucasians, such as Austrian (P = 0.0127; 95% CI: 2.94% to 18.725%), Spanish (P = 0.0007; 95% CI: 6.447% to 20.646%), and Sardinian (P = 0.0012; 95% CI: 3.701% to 17.374%) populations, which was followed by Brazilian (P < 0.0001; 95% CI: 8.003% to 20.345%), African American (P = 0.0001; 95% CI: 5.61% to 19.042%) and UAE (P = 0.0006; 95% CI: 5.408% to 19.399%) populations. However, it showed significantly lower frequency when compared with Asian populations such as Chinese Han (P < 0.0001; 95% CI: 28.53% to 44.304%), Korean (P < 0.0001; 95% CI: 21.044% to 36.8%), Japanese (P < 0.0001; 95% CI: 19.696% to 33.353%) and Thai (P < 0.0001; 95% CI: 20.949% to 37.058%).

The functional consequence of the non-synonymous SNP in the first exon of CYP2D6 gene has predicted by PolyPhen-2. It has been proven that 100C>T (rs1065852; p.Pro34Ser) is the SNP with possibly damaging the enzyme activity with a score of 0.946 (HumDiv) and 0.687 (HumVar). The results of this analysis have shown in Figure 1.

5. DISCUSSION

The CYP2D6 gene is located on chromosome 22, nearby two pseudogenes CYP2D7 and CYP2D8 that share 95% sequence homology with CYP2D6. The presence of the highly similarly closely located pseudogenes with detrimental mutations have led to the formation of many of the variant CYP2D6 alleles of most commonly encode defective gene products.

In this study, genotyping of the CYP2D6 gene in the Bosnian sample identified 2.75% of the Bosnians with more than two active CYP2D6 gene copies. The individuals with multiple CYP2D6 gene copies metabolize drugs more rapidly and therapeutic plasma levels will not be achieved at ordinary drug dosages. The CYP2D6 gene duplication/multiplication, resulting from CYP2D rearrangements (CYP2D6*xn), are present with allele frequencies of 1–10% in Caucasian individuals (20). The frequency of UMs varies from 1–2% in Asians to 29% in some African populations (21).

The CYP2D6 100C>T allele frequency in the Bosnian population (15.56.0%) was similar to frequencies of this allele in Ethiopian, Turkish and Uygur but significantly lower when compared with East Asian populations. In contrast, the frequencies of 100C>T variant in the Bosnian were significantly higher when compared with the other Caucasians, such as Austrian, Spanish, and Sardinian populations, which was followed by Brazilian, African American and UAE populations. The CYP2D6 100C>T decreased-function allele frequencies differ considerably between populations. The allele is lowest in Oceanians and Europeans and highest in East Asians, averaging 1.6, 2.6 and 45%, respectively (22). It has been confirmed CYP2D6 100C>T (rs1065852; p.Pro34Ser) is the SNP with possibly damaging the enzyme activity. This SNP can occur on 19 different haplotypes (CYP2D6*4, *10, *14, *36, *37, *47, *49, *52, *54, *56, *57, *64, *65, *68, *72, *94, *95, *100 and *101).

Single nucleotide polymorphisms (SNPs) may help predict an individual's response to certain drugs, susceptibility to environmental factors such as toxins, and risk of developing particular diseases. CYP2D6 metabolizes a number of commonly prescribed drugs. Variations in the gene encoding this enzyme have been associated with individual differences in drug metabolism rates. The variant CYP2D6 alleles can be classified into categories, which cause abolished, decreased, normal, increased or qualitatively altered catalytic activity. The CYP2D6 100C>T allelic variant is part of both the reduced function CYP2D6*10 haplotype and the non-functional CYP2D6*4 haplotype. IMs, with two reduced function or
one reduced and one non-functional allele, are not able to metabolize CYP2D6 substrates like EMs.

6. CONCLUSIONS

The CYP2D6 gene duplication/multiplication frequency found in the Bosnian population was similar to the frequencies of this allele in other Caucasians (2.73%). The gene duplication is the result of inheritance of more than two copies of the fully functional CYP2D6 alleles and is an autosomal dominant trait.

The frequency of the CYP2D6 100C>T variant, with possibly damaging function, in the Bosnian population (15.56%) was more similar to the frequencies of this allele in Ethiopian, Turkish and Uygur. In contrast, the frequencies of 100C>T variant in the Bosnian were significantly higher when compared with the other Caucasians, such as Austrian, Spanish, and Sardinian populations, which was followed by Brazilian, African American and UAE populations but significantly lower when compared with Asians.

The genetic polymorphism is associated not only with diversity within and among populations, but also with individual responses to medication and susceptibility to diseases and represent basis for individualized medical treatment and drug genomics studies.

Acknowledgments: This project was financially supported by grants from the Federal Ministry of Education and Science (Federation of Bosnia and Herzegovina) (grant numbers 05-39-3627-1/14 and 0101-1877-6/16).

Author's contribution: The author was included in all phases of preparation this article and gave final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest: There are no conflicts of interest.

Financial support and sponsorship: None.

REFERENCES

1. Ingelman-Sundberg M. Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future. Trends Pharmacol Sci. 2004 Apr; 25(4): 193-200. doi: 10.1016/j.\tips.2004.02.007.

2. Kimura S, Umeno M, Skorda RC, Meyer UA, Gonzalez FJ. The human debrisoquine 4-hydroxylase (CYP2D) locus: sequence and identification of the polymorphic CYP2D6 gene, a related gene, and a pseudogene. Am J Hum Genet. 1989 Dec; 45(6): 889-904.

3. Mejierman I, Sanderson LM, Smits PH, Beijnen JH, Schellens JH. Pharmacogenetic screening of the gene deletion and duplications of CYP2D6. Drug Metab Rev. 2007; 39(1): 45-60. doi: 10.1080/3602530600952206.

4. 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. Clin Pharmacol Ther. 2008 Feb; 83(2): 234-242. doi: 10.1038/sj.cpt.6100406.

5. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res. 1988 Feb; 16(3): 1215.

6. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010 Apr; 7(4): 248-249. doi: 10.1038/nmeth0410-248.

7. Beer B, Erb R, Pitterl F, Niederstätter H, Maroñas O, Gesteira A, et al. CYP2D6 genotyping by liquid chromatography-electrospray ionization mass spectrometry. Anal Bioanal Chem. 2011 Jun; 400(8): 2361-2370. doi: 10.1007/s00216-010-4597-4.

8. Menoyo A, del Rio E, Baijet M. Characterization of variant alleles of cytochrome CYP2D6 in a Spanish population. Cell Biochem Funct. 2006 Sep-Oct; 24(5): 381-385. doi: 10.1002/cbf.1258.

9. Falzoi M, Pira L, Lazzari P, Pani L. Analysis of CYP2D6 allele frequencies and identification of novel SNPs and sequence variations in Sardinians. ISRN Genetics. 2013; 2013: 1-10. http://dx.doi.org/10.5402/2013/204560.

10. Friedrich DC, Genjo JP, Sortica VA, Suarez-Kurtz G, de Moraes ME, Pena SDI, et al. Distribution of CYP2D6 alleles and phenotypes in the Brazilian population. PLoS One. 2014 Oct; 9(10): e110691. doi: 10.1371/journal.pone.0110691.

11. Cai WM, Nikoloff DM, Pan RM, de Leon J, Fanti P, Fairchild M, et al. CYP2D6 genetic variation in healthy adults and psychiatric African-American subjects: implications for clinical practice and genetic testing. Pharmacogenomics J. 2006 Sep-Oct; 6(5): 343-350. doi: 10.1038/sj.tpj.6500378.

12. Akulliu E, Persson I, Bertilsson L, Johansson L, Rodrigues F, Ingelman-Sundberg M. Frequent distribution of ultrarapid metabolizers of debrisoquine in an ethiopian population carrying duplicated and multiduplicated functional CYP2D6 alleles. J Pharmacol Exp Ther. 1996 Jul; 278(1): 441-446.

13. Qumsieh RY, Ali BR, Abdulrazzaq YM, Osmann O, Akawi NA, Bastaki SMA. Identification of new alleles and the determination of allele and genotypes frequencies at the CYP2D6 gene in Emiratis. Zanger UM, ed. PLoS One. 2011; 6(12): e28943. doi: 10.1371/journal.pone.0028943.

14. Serin A, Canan H, Alper B, Gulmen M. The frequencies of mutated alleles of CYP2D6 gene in a Turkish population. Forensic Sci Int. 2012 Oct; 222(1-3): 332-334. doi: 10.1016/j.forsciint.2012.07.012.

15. He X, He N, Ren L, Ouyang Y, Zhang N, Ma Y, et al. Genetic polymorphisms analysis of CYP2D6 in the Uygur population. BMC Genomics. 2016 May; 17: 409. doi: 10.1186/s12866-016-2719-x.

16. Qin S, Shen L, Zhang A, Xie J, Shen W, Chen L, et al. Systematic polymorphism analysis of the CYP2D6 gene in four different geographical Han populations in mainland China. Genomics. 2008 Sep; 92(3): 152-158. doi: 10.1016/j.ygeno.2008.05.004.

17. Lee SY, Sohn KM, Ryu JY, Yoon YR, Shin JG, Kim JW. Sequence-based CYP2D6 genotyping in the Korean population. Ther Drug Monit. 2006 Jun; 28(3): 382-387. doi: 10.1097/01.tdm.0000211823.80854.db.

18. Ota T, Kamada Y, Hayashida M, Iwao-Koizumi K, Murata S, Kinoshita K. Combination analysis in genetic polymorphisms of drug-metabolizing enzymes CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A5 in the Japanese population. Int J Med Sci. 2015 Jan; 12(1): 78-82. doi: 10.7150/ijms.10263.

19. Suvannasri P, Thongnoppakhun W, Pramothen P, Assawama-kin A, Limwongse C. Combination of multiplex PCR and DH-PLC-based strategy for CYP2D6 genotyping scheme in Thais. Clin Biochem. 2011 Sep; 44: 1144-1152. doi: 10.1016/j.clinbiochem.2011.06.985.

20. Ingelman-Sundberg. Genetic polymorphisms of cytochrome P4502D6 (CYP2D6): clinical consequences, evolutionary aspects, and functional diversity. Pharmacogenomics J. 2005; 5: 6-13. doi: 10.1038/sj.tpj.6500285.

21. Ji L, Pan S, Marti-Jaun J, Hänseler E, Rentsch K, Hersberger M. Single-step assays to analyze CYP2D6 gene polymorphisms in Asians: allele frequencies and a novel *14B allele in mainland Chinese. Chem. Clin 2002 Jul; 48(7): 983-988.

22. Gaedigk A, Sangkuhl K, Whirl-Carrillo M, Klein T, Leeder JS. Prediction of CYP2D6 phenotype from genotype across world populations. Genet Med. 2017 Jan; 19(1): 69-76. doi: 10.1038/gim.2016.80.