CYP2D6 Polymorphism of Opiate Addicts and Contributions to Forensic Sciences

Opiyat Bağımlılarında *CYP2D6* Polimorfizmi ve Adli Bilimlere Katkıları

Münevver AÇIKKOL,[a]
Selda MERCAN[a]

[a]İstanbul University,
Institute of Forensic Sciences,
İstanbul

Geliş Tarihi/*Received:* 17.08.2010
Kabul Tarihi/*Accepted:* 29.01.2011

Yazışma Adresi/*Correspondence:*
Selda MERCAN
İstanbul University,
Institute of Forensic Sciences, İstanbul,
TÜRKİYE/TURKEY
mercans@istanbul.edu.tr

ABSTRACT Objective: The *CYP2D6* enzyme shows a wide range of polymorphisms. It is responsible for the metabolism of substances such as opiates that have a great importance in forensic sciences. However, some mutations damage the activity of this enzyme and cause interruption in metabolism of the drugs which may lead to unexpected adverse drug reactions, even to death. Although many studies have dealt with *CYP2D6* polymorphism, there is no research concerning *CYP2D6* enzyme polymorphism and opiate dependence in our country. **Material and Methods:** We aimed to investigate the single nucleotide polymorphisms of *CYP2D6*3*, *4 and *6 alleles. Forty nine opiate addicts and 34 control samples were studied using tetra-primer polymerase chain reaction (PCR) technique in a single tube and by dividing the protocol into two steps. **Results:** *CYP2D6*4* and *CYP2D6*6* alleles frequencies were determined as 0.082 and 0.031, respectively, however no significant result was obtained for *CYP2D6*3*. Allele and genotype frequencies of *CYP2D6*4* and *6 for both opiate addicts and control group were in concordance with the Hardy-Weinberg equation. **Conclusion:** We suggest that detection of *CYP2D6* polymorphism may clarify the sudden-unexpected deaths due to intoxication of opiate addicts and some negative autopsy cases related to medical or illicit drugs.

Key Words: Pharmacogenetics; cytochrome P-450 *CYP2D6*; opioid-related disorders; drug toxicity

ÖZET Amaç: *CYP2D6* enzimi çok çeşitli polimorfizmleri gösterebilmektedir ve opiyatlar gibi adli tıp biliminde büyük önemi olan maddelerin metabolizmasından sorumludur. Ayrıca bazı mutasyonlar bu enzimin aktivitesini bozar ve alınan ilaçların metabolizmasını kesintiye uğratırlar ki bu da beklenmeyen advers ilaç reaksiyonlarına hatta ölüme yol açabilir. *CYP2D6* polimorfizmi ile ilgili birçok çalışma olsa da bizim ülkemizde *CYP2D6* enzim polimorfizmi ve opiyat bağımlılığı ile ilgili çalışma bulunmamaktadır. **Gereç ve Yöntemler:** *CYP2D6*3*, *4 ve *6 allellerindeki tek nükleotid polimorfizmlerini araştırmayı amaçladık. 49 opiyat bağımlısı ve 34 kontrolden alınan örnek tetra- primer polimeraz zincir reaksiyonu (PCR) tekniği ile tek tüpte ve protokolü iki aşamaya bölerek çalışıldı. **Bulgular:** *CYP2D6*4* ve *CYP2D6*6* allel sıklıkları sırasıyla 0.082 ve 0.031 olarak belirlendi, bununla birlikte *CYP2D6*3* alleli ile ilgili hiçbir anlamlı sonuç elde edilemedi. Hem opiyat bağımlıları hem de kontrol grubu için *CYP2D6*4* ve *6'nın allel ve genotip sıklıkları Hardy-Weinberg denklemi ile uyum göstermektedir. **Sonuç:** Biz *CYP2D6* polimorfizminin saptanmasının opiyat bağımlılarının zehirlenmelerine bağlı ani ve beklenmedik ölümleri açıklayabileceğini ve tıbbi ve yasadışı ilaçlarla ilişkili negatif otopsi olgularını açıklığa kavuşturabileceğini düşündük.

Anahtar Kelimeler: Farmakogenetik; sitokrom P-450 *CYP2D6*; opiyat ilişkili bozukluklar; ilaç toksisitesi

doi:10.5336/medsci.2010-20682

Turkiye Klinikleri J Med Sci 2011;31(6):1418-24

The Cytochrome P450 (CYP450) multi-enzyme system plays a key role in the action and elimination process of many drugs. In clinical pharmacology, there are five important CYP450 enzymes which are encoded by separate genes. These enzymes are CYP1A2, CYP2C19, *CYP2D6*, CYP2E1 and CYP3A4. CYP450, is different for its amino acid composition, and so its substrate characteristics. *CYP2D6*, the most commonly investigated CYP450 enzyme, is the major enzyme catalyzing the biotransformation of xenobiotics, tricyclic antidepressants, most of selective serotonin reuptake inhibitors (SSRIs), antiarrhythmics, neuroleptics, beta-blockers and opioids.[1] Some drugs metabolized by *CYP2D6* enzyme and have forensic importance are given in Table 1. Most of them might be encountered in clinical and forensic cases.[2] Polymorphisms of *CYP2D6* gene alter the activation of the enzyme function and also affect the metabolisms of xenobiotics and drugs. Variations occur in genes of this enzyme and lead to different phenotypes that can be classified into four groups as PM (poor metabolizer), IM (intermediate metabolizer), EM (extensive metabolizer), and UM (ultra rapid metabolizer).[3-5]

CYP2D6 is a polymorphic gene including nine exons and eight introns (GenBank Accession No. M33388).[6] Alleles may have one or more single nucleotide polymorphisms (SNPs) such as deletions, insertions, duplications and multi duplications.[7] Approximately 5% of Europeans and 1% of Orientals lack *CYP2D6* activity and these individuals are known as poor metabolizers.[1] Ninety five percent of European poor metabolizers can be identified by screening of *CYP2D6*3* (2549 A>del), *CYP2D6*4* (1846 G>A), *CYP2D6*5* (*CYP2D6* del) and *CYP2D6*6* (1707 T>del) alleles.[8-11]

Variability of a drug response in individuals is a major problem because it leads to therapeutic failure and adverse drug reactions (ADRs) in clinical practice. Therefore, importance of *CYP2D6* polymorphisms must be emphasized for forensic pharmacogenetic.[12] A case study carried out by Levo et al. concerned with post-mortem SNP analysis of *CYP2D6* gene and revealed correlation between genotype and opioid drug tramadol metabolite levels in blood.[13] Bailey et al. studied genetic polymorphism of *CYP2D6* enzyme involved in the metabolism of dextromethorphan which is commonly used as an indicator of *CYP2D6* genetic polymorphism. In this study, a toddler was found dead after a therapeutic dose of dextromethorphan, without any evidence of a systemic disease. Intentional or unintentional overdose was not found concerning the cause of death, which is a typical example for forensic pharmacogenetics.[14] Chiurillo et al. investigated *CYP2D6* variation in Venezuelan population to conduct postmortem pharmacogenetic analysis in case of drug intoxications.[15] In autopsy cases such as death due to suspected poisoning, authors reported that genotyping of variant CYP-isoform gene could help to determine the causes of death and to increase the accuracy of forensic identification of the cadaver.[16] In Sweden, the groups of deaths caused by intoxication of pharmaceuticals or illegal drugs were founded upon *CYP2D6* genotyping.[17]

Opiate addiction is one of the most important issues of drug dependence in the forensic toxicology in our country as well as all over the world.

TABLE 1: Some drugs metabolized by polymorphic *CYP2D6* enzyme (Davies and Nutt 2004).

	Drug Group	Substrates
CYP2D6	Antidepressants	Paroxetine, Fluoxetine, Citalopram, Sertraline, Venlafaxine, Amitriptyline, Clomipramine, Desipramine, Imipramine, Nortriptyline
	Antipsychotics	Chlorpromazine, Haloperidol, Thioridazine, Zuclopenthixol, Perphenazine, Risperidone,
	Miscellaneous	Bupropion, β-blockers, Propanolol, Metoprolol, Timolol, Dexfenfluramine, Ecstasy, Opioids, Codeine, Hydrocodone, Dihydrocodeine, Tramadol

Most of drug overdose-related deaths are caused by opioids such as morphine, codeine, dextromethorphan, ethylmorphine and tramadol. An opiate addict with impaired *CYP2D6* enzyme may show unexpected ADRs with ordinary does that may result in death due to overdose.[18-20]

As mentioned above, poor metabolizers have higher levels of a number of drugs in their plasma, which may increase the risk of adverse and toxic effects.

In this study, single nucleotide polymorphisms of *CYP2D6*3, *4* and **6* alleles on opiate addicts were investigated with allele-specific tetra primer polymerase chain reaction (PCR).

MATERIAL AND METHODS

The study groups consisted of 49 opiate addicts (32 males and 17 females) who admitted to Istanbul Balikli Rum Anatolian Clinic for treatment of opiate addiction (like morphine, opium gum, aldolane, phentanyl) and 34 unrelated, non-addict volunteers (11 males and 23 females), as the control group. The study protocol was approved by the Ethics Committee of The Institute of Forensic Science in Istanbul University, and written informed consents were obtained from the volunteers.

Five mL of venous blood samples were collected into EDTA tubes and DNA was isolated with DNA extraction kit (QIAmp® Mini Kit QIAGEN, Germany). The isolates were checked with 2% agarose gel electrophoresis and stored at -20°C until use. HPLC grade primers described by Hersberger (Table 2) were purchased from Thermo-electron GmbH (Germany).[21] All PCR reagents and ladders were purchased from Fermentas (Lithuanian), chemical reagents were purchased from Sigma-Aldrich (Germany). The PCR amplifications were carried out by Gold-plated GeneAmp®PCR 9700 system, Applied Biosystems (Canada, USA).

The method previously described by Hersberger et al. was used with some modifications to determine the frequencies of *CYP2D6*3* (2549 A>del), **4* (1846 G>A) and **6* (1707 T>del) alleles with tetra primer PCR.[21] The first pair of primers (for example P_1 and P_2 via *CYP2D6*3*) amplified the first fragment which was the template of the second pair of primers. The first amplicon was also the control of the PCR. According to the mutation, one of the second primers (P_3 or P_4) was combined with one of the first step primers (P_1 or P_2). The length of base pair was determined with agarose and polyacrylamide gel electrophoresis.

TABLE 2: Primer sequences used in the study (Hersberger 2000).

Allele	Name of Primer	5'-3' primer sequence
*CYP2D6*3*	P1	GCG GAG CGA GAG ACC GAG GA
	P2	GGT CCG GCC CTG ACA CTC CTT CT
	P3	TCC CAG GTC ATC CT
	P4	GCT AAC TGA GCA CG
*CYP2D6*4*	P5	TCC CAG CTG GAA TCC GGT GTC G
	P6	GGA GCT CGC CCT GCA GAG ACT CCT
	P7	TCT CCC ACC CCC AA
	P8	CGA AAG GGG CGT CC
*CYP2D6*6*	P5	TCC CAG CTG GAA TCC GGT GTC G
	P6	GGA GCT CGC CCT GCA GAG ACT CCT
	P9	GTC GCT GGA GCA GG
	P10	TCC TCG GTC ACC CA

In the final PCR mixture of *CYP2D6*3, *4* and **6* for tetra-primer PCR method in single tube, the volume was 30 µL with 2.5 µL (10X) Taq buffer, 2.5 µL (25 mM) $MgCl_2$, 1µL (1.25 mM) dNTPmix, 0.5 µL BSA, 1 µL primer (30 pmol) (for the first fragment) per allele, 4 µL primer (for the second fragment) per allele, 1 µL Taq (5 U/µL), 2.5 µL (~62 ng/µL) template DNA and 10 µL distilled water. PCR conditions were as follows; 10 min at 94°C, and for the first 30 cycles, 0.5 min at 95°C, 0.5 min at 63°C, 1 min at 72°C, for the second 30 cycles, 0.5 min at 95°C, 0.5 min at 53°C, 1 min at 72°C and the reaction was finished with 7 min extension at 72°C.

On the other hand, the method of Hersberger et al. was modified by dividing the PCR procedure. Once the first pair of primer was used and the control fragment was generated in an individual step with first 30 cycles under the same conditions, the control fragment was used as template DNA for the second annealing related under allele specific PCR with the same PCR conditions for second 30 cycles. In this modified method, the amounts of reagents

and enzymes were the same as the method described above.

Separation of PCR products was performed with 4% polyacrylamide gel electrophoresis for 2h visualized by $AgNO_3$ (for *CYP2D6*3* allele) and 2% agarose gel electrophoresis for 50 min visualized by ethidium bromide under UV transilluminator (for *CYP2D6*4* and **6* alleles). Statistical analyses were performed using Hardy Weinberg equation and statistical significance of opiate addicts-control associations was evaluated using Fisher's exact test.[22] A p value smaller than 0.05 was considered as statistically significant.

RESULTS

This study is the first one that determined the frequency of *CYP2D6*3, *4* and **6* alleles in opiate addicts in Istanbul, Turkey. Tetra primer PCR results for three alleles and amplified fragments are given in Figure 1. The frequency of *CYP2D6*4* allele was 0.082 and *CYP2D6*6* allele was 0.031 for opiate addicts, whereas they were 0.103 and 0.015 for then control group, respectively (Table 3). There were no mutant alleles result of for *CYP2D6*3* in two groups. In modified tetra primer PCR method in single tube, both control fragments and allele-specific fragments were seen in the same PCR product.

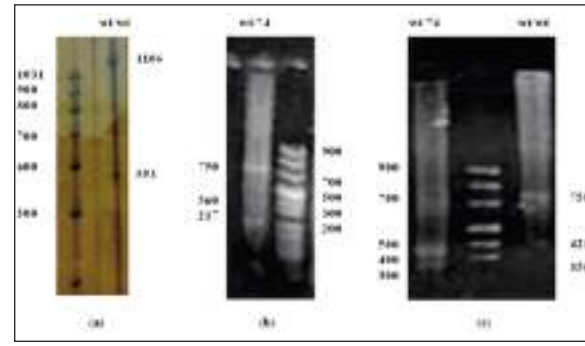

FIGURE 1: Analysis of alleles by 4% polyacrylamide (a) and 2% agarose gel electrophoresis (b and c).
a. *CYP2D6*3*, 1106 bp is control, 553 bp is wt (wild type) fragment.
b. *CYP2D6*4*, 750 bp is control, 560 bp is wt and 217 is mutant fragment.
c. *CYP2D6*6*, 750 bp is control, 421 bp is wt, 356 bp is mutant fragment.

On the other hand, in the first divided step of the modified method, only control fragments were seen; in the second step, allele-specific fragments were amplified and checked by electrophoresis. The results showed the same genotypes with tetra primer PCR protocol. Allele and genotype frequencies of *CYP2D6*4* and **6* for both opiate addicts and control group were parallel to the Hardy-Weinberg equation. There was no significant difference between cases and control subjects according to Fisher's exact test. Observed and predicted genotypes of study and control groups and p values are given in Table 4.

TABLE 3: *CYP2D6* allele numbers and frequencies of opiate addicts and control groups.

Allele	Opiate addicts		Control group	
	Mutant allele /Total allele numbers		Mutant allele /Total allele numbers	
	(n= 98)	Mutant Allele frequencies	(n= 68)	Mutant Allele frequencies
*3	0/98	-	0/68	-
*4	8/98	0.082	7/68	0.103
*6	3/98	0.031	1/68	0.015

TABLE 4: Observed and predicted genotypes of subjects and p values of X2 test.

Subjects	N	Observed (expected) *CYP2D6 *4* genotypes			Observed (expected) *CYP2D6 *6* genotypes		
		*4/*4	*4/wt[b]	wt/wt	*6/*6	*6/wt	wt/wt
Opiate Addicts	49	0 (0.0)	8 (8.86)	41 (40.14)	0 (0.0)	3 (2.36)	46 (46.64)
Control	34	0 (0.0)	7 (6.14)	27 (27.86)	0 (0.0)	1 (1.64)	31 (32.36)
p[a]			0.246			0.443	

[a] degrees of freedom value: 1 ($p < 0.05$) [b] wild type.

DISCUSSION

Drug metabolizing enzymes show genetic variations leading to inter-individual differences in drug response and consequently to ADRs, high or low drug levels at and ordinary dose.[23,24] Drug metabolism may be affected not only by genetic variations but also by metabolic drug interactions, enzyme inhibitions and inductions, drug-drug interactions, age, renal or liver malfunction, diseases, alcohol, smoking, diet, etc.[5,18]

Drug therapies are frequently associated with great inter- and intra-individual differences in therapeutic response because of the polymorphisms. This variability may result in diminished clinical response or conversely, increased incidence of adverse drug reactions. Investigation of *CYP2D6* polymorphism is important for the ability to predict drug response that would allow individualized pharmacotherapy, maximize the chance of optimal drug choice for each patient and consequently, could offer savings in both time and cost of case and substantially improve the patient's long-term prognosis. Many drugs used clinically, such as cardiovascular and psychoactive (antidepressant, neuroleptic) drugs and some of opiates are metabolized by *CYP2D6*. The lack of *CYP2D6* activity in PMs may lead to excessively high plasma concentrations of drugs that are substrates for this CYP enzyme. Consequently intoxication and death are important problems for the forensic toxicology. Most frequently, the overdose cases are caused by opiates which might result in death. An opiate addict with impaired *CYP2D6* enzyme may show unexpected adverse drug reactions at an ordinary dose that cause overdose death. Forensic toxicology can contribute substantially to the interpretation and determination of the causes and manner of death. Most fatal intoxications are suicidal overdoses, but recently, chronic high dose has attracted increased interest. A high ratio between the parent drug and the metabolite could be interpreted not only as an overdose but could also be the result of a reduced metabolism of the parent drug caused by enzymes such as *CYP2D6* and CYP2C19.[19,20]

Candiotti et al. showed that *CYP2D6* UM appear to require less morphine in the acute postoperative period compared to other *CYP2D6* metabolizer groups, and recommended further studies to understand the role of the different *CYP2D6* metabolizers in endogenous morphine synthesis and pain modulation.[25]

Eap et al. found a significant association between therapeutic response and (R)-methadone blood concentrations and reported that more studies were needed to examine the influence of the ultrarapid metabolizer status.[26]

Levo et al. studied the effect of genetic variation of drug metabolizing enzymes on correlation of parent drug (tramadol) to metabolite ratio in postmortem blood. Authors determined two major genetic rearrangements and 18 SNPs along the *CYP2D6* gene.[13] Ogawa et al. determined the genotype frequencies of *CYP1A2, CYP2D6, CYP2E1, CYP2C9* and *CYP2C19* in 196 Japanese individuals to evaluate their forensic usefulness and carried out forensic identification of cadaver by genetic information of CYP-isoform genes.[16] Riccardi et al. carried out an investigation on the polymorphism of *CYP2D6*3, *4, *5, *6* alleles, emphasizing the use of pharmacogenetic analysis on postmortem samples for forensic purposes. Another utility of that research was the medical liability to ADRs, fatal drug and/or special pesticide poisonings.[27] Bailey et al. reported death of a toddler after a therapeutic dose of dextromethorphan. The possible explanation was PM for *CYP2D6* according to redistribution studies and autopsy results.[14] Lack of functional enzymes and polymorphisms in the *CYP2D6* gene have been studied extensively, 95% of European PMs can be identified by screening for the *CYP2D6*3, *4, *5, *6.*[1,10,11] Tamminga et al. carried out a retrospective study to assess the influence of polymorphic drug metabolism in hospitalized psychiatric patients in Netherlands, and the obtained results for *CYP2D6* were 2.5% for UMs, 8.3% for PMs. Authors emphasized that indications for a higher incidence of ADRs were observed in PMs and pharmacy records might be useful to detect differences related to polymorphic metabolism.[28] Scordo et al. determined genotypes of a random Italian population in order to compare *CYP2C9, CYP2C19* and *CYP2D6* allele frequencies. They found 3.4%

PMs and 8.3% UMs for *CYP2D6.*[29] Aynacioglu et al. investigated CYP450 enzymes 2C19 and 2D6 in Turkish and German population. *CYP2D6*3* allele was not found in any of 404 subjects and **4, *6* allele frequencies were found as 0.113 and 0.007, respectively.[30] *CYP2D6*3* allele was not found and other two allele frequencies were different from the present study. The difference of the frequencies may be due to the different numbers of subjects. Herken et al. carried out a study in Gaziantep, Turkey for psychiatric drugs metabolized by *CYP2D6* enzyme. The result of the *CYP2D6*4* frequency was found as 0.081, which was similar with to our results.[31] Koseler et al. determined the most common mutated allele as *CYP2D6*4* gene in a Turkish population with 100 unrelated subjects living in Bursa region. They reported 0.21 frequency for *CYP2D6*4* allele which was much higher than the data of the present study.[32] Aydin et al. investigated the allele and genotype frequencies of *CYP1A1*2A, CYP2D6*3* and **4* in the Turkish population, and report different frequencies compared to our study, as 0.15 for *CYP2D6*4* and 0.025 for *CYP2D6*3* alleles.[33]

It can be concluded that all of these studies carried out in our country show variations for allele frequencies according to regions and number of subjects. With regard to our study, the main aim was to compare *CYP2D6*3, *4* and **6* allele frequencies between opiate addicts and a control group.

CONCLUSION

In the present study, we used tetra primer single tube PCR assay, the method reduced the risk for handling errors and contamination, and facilitated genotyping of *CYP2D6* alleles except regulation and optimization of primer concentrations. In conclusion, we presented a study for genotypes of *CYP2D6*3, *4* and **6* alleles for the first time in opiate addicts in Turkey. It can be concluded that the genetic polymorphism of *CYP2D6* among opiate addicts may cause sudden unexpected death or overdose. We recommend a forensic pharmacogenetic investigation in unintentional intoxications and/or some negative autopsy cases with licit or illicit drugs metabolized by *CYP2D6* enzyme. Although there was no significant difference between opiate addicts and the controls in this study, another study may be contucted an a greater patient population.

Acknowledgment

We would like to thank the Research Fund of Istanbul University for support of this work with a Project T-649/17032005, Anatolian Clinical of Istanbul Balikli Rum Hospital and Prof. Ersi Abaci Kalfoglu. Istanbul University deemed Selda Mercan worthy of award of "Scientist of the Year, 2006" with this project. (This acknowledgment part is added later and is not written in the print copy of the journal but it is just existing on the online version since 01/24/2012)

REFERENCES

1. Daly AK. Pharmacogenetics of the major poly1. Daly AK. Pharmacogenetics of the major polymorphic metabolizing enzymes. Fundam Clin Pharmacol 2003;17(1):27-41.
2. Davies SJC, Nutt D. Pharmacokinetics for psychiatrists. Psychiatry 2004;3(1):5-9.
3. Zanger UM, Raimundo S, Eichelbaum M Cytochrome P450 2D6: overview and update on pharmacology, genetics, biochemistry. Naunyn Schmiedebergs Arch Pharmacol 2004;369(1):23-37.
4. Bock KW, Schrenk D, Forster A, Griese EU, Mörike K, Brockmeier D, et al. The influence of environmental and genetic factors on CYP2D6, CYP1A2 and UDP-glucuronosyltransferases in man using sparteine, caffeine, and paracetamol as probes. Pharmacogenetics 1994;4(4):209-18.
5. Tanaka E. Polymorphism of drug metabolizing enzymes in humans. Sepsis 2001;4(3): 247-54.
6. Kimura S, Umeno M, Skoda 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;45(6):889-904.
7. Daly AK, Brockmöller J, Broly F, Eichelbaum M, Evans WE, Gonzalez FJ, et al. Nomenclature for human CYP2D6 alleles. Pharmacogenetics 1996;6(3):193-201.
8. Marez D, Legrand M, Sabbagh N, Lo Guidice JM, Spire C, Lafitte JJ, et al. Polymorphism of the cytochrome P450 CYP2D6 gene in a European population: characterization of 48 mutations and 53 alleles, their frequencies and evolution. Pharmacogenetics 1997;7(3):193-202.
9. Roberts RL, Kennedy MA. Rapid detection of common cytochrome P450 2D6 alleles in Caucasians. Clin Chim Acta 2006;366(1-2):348-51.
10. Sachse C, Brockmöller J, Bauer S, Roots I. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. Am J Hum Genet 1997;60(2):284-95.

11. Leathart JB, London SJ, Steward A, Adams JD, Idle JR, Daly AK. CYP2D6 phenotype-genotype relationships in African-Americans and Caucasians in Los Angeles. Pharmacogenetics 1998;8(6):529-41.

12. Meyer UA. Pharmacogenetics and adverse drug reactions. Lancet 2000;356(9242):1667-71.

13. Levo A, Koski A, Ojanperä I, Vuori E, Sajantila A. Post-mortem SNP analysis of CYP2D6 gene reveals correlation between genotype and opioid drug (tramadol) metabolite ratios in blood. Forensic Sci Int 2003;135(1):9-15.

14. Bailey B, Daneman R, Daneman N, Mayer JM, Koren G. Discrepancy between CYP2D6 phenotype and genotype derived from post-mortem dextromethorphan blood level. Forensic Sci Int 2000;110(1):61-70.

15. Chiurillo MA, Grimán P, Morán Y, Camargo ME, Ramírez JL. Analysis of CYP2D6 gene variation in Venezuelan population: Implications for forensic toxicology. Forensic Science International: Genetics Supplement Series 2009;2(1):483-4.

16. Ogawa K, Suno M, Shimizu K, Yoshida M, Awaya T, Matsubara K, et al. Genotyping of cytochrome p450 isoform genes is useful for forensic identification of cadaver. Leg Med (Tokyo) 2003;5(3):132-8.

17. Zackrisson AL, Holmgren P, Gladh AB, Ahlner J, Lindblom B. Fatal intoxication cases: cytochrome P450 2D6 and 2C19 genotype distributions. Eur J Clin Pharmacol 2004; 60 (8):547-52.

18. Pirmohamed M, Park BK. Cytochrome P450 enzyme polymorphisms and adverse drug reactions. Toxicology 2003;192(1):23-32.

19. Druid H, Holmgren P, Carlsson B, Ahlner J. Cytochrome P450 2D6 (CYP2D6) genotyping on postmortem blood as a supplementary tool for interpretation of forensic toxicological results. Forensic Sci Int 1999;99(1):25-34.

20. Verrecas M, Knaepen K, Gilissen A, Cassiman J-J, Decorte R. Forensic toxicology: development of an SNP-assay for genotyping CYP2D6 and CYP2C19 variants. Int Congr 2004;1261:583-5.

21. Hersberger M, Marti-Jaun J, Rentsch K, Hänseler E. Rapid detection of the CYP2D6*3, CYP2D6*4, and CYP2D6*6 alleles by tetra-primer PCR and of the CYP2D6*5 allele by multiplex long PCR. Clin Chem 2000;46(8 Pt 1):1072-7.

22. Flockhart DA, Bertilsson L. Clinical pharmacogenetics. In: Atkinson AJ, Abernethy DR, Daniels CE, Dedrick RL, Markey SP, eds. Principles of Clinical Pharmacology. 2nd ed. New York: Academic Press; 2007. p.179-95.

23. Meyer UA, Zanger UM. Molecular mechanisms of genetic polymorphisms of drug metabolism. Annu Rev Pharmacol Toxicol 1997; 37:269-96.

24. Ingelman-Sundberg M. Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future. Trends Pharmacol Sci 2004;25(4):193-200.

25. Candiotti KA, Yang Z, Rodriguez Y, Crescimone A, Sanchez GC, Takacs P, et al. The impact of CYP2D6 genetic polymorphisms on postoperative morphine consumption. Pain Med 2009;10(5):799-805.

26. Eap CB, Broly F, Mino A, Hämmig R, Déglon JJ, Uehlinger C, et al. Cytochrome P450 2D6 genotype and methadone steady-state concentrations J Clin Psychopharmacol 2001; 21(2):229-34.

27. Riccardi LN, Bini C, Ceccardi S, Trane R, Luiselli D, Pelotti S. CYP2D6 polymorphism studies: How forensic genetics helps clinical medicine. Forensic Science Int. Genetics Supplement Series 2009;2(1):485-6.

28. Tamminga WJ, Wemer J, Oosterhuis B, de Boer A, Vranckx S, Drenth BF, et al. Polymorphic drug metabolism (CYP2D6) and utilisation of psychotropic drugs in hospitalised psychiatric patients: a retrospective study. Eur J Clin Pharmacol 2003;59(1):57-64.

29. Scordo MG, Caputi AP, D'Arrigo C, Fava G, Spina E. Allele and genotype frequencies of CYP2C9, CYP2C19 and CYP2D6 in an Italian population. Pharmacol Res 2004; 50(2): 195-200.

30. Aynacioglu AS, Sachse C, Bozkurt A, Kortunay S, Nacak M, Schröder T, et al. Low frequency of defective alleles of cytochrome P450 enzymes 2C19 and 2D6 in the Turkish population. Clin Pharmacol Ther 1999;66 (2): 185-92.

31. Herken H, Aynacıoğlu S, Esgi K, Vırıt O. [The frequencies on poor and ultrarapid metabolizers of cytochrome P450-2D6 in psychiatric patients]. Turk Psikiyatri Derg 2001; 12(2):83-8.

32. Koseler A, Ilcol YO, Ulus IH. Frequency of mutated allele CYP2D6*4 in the Turkish population. Pharmacology 2007;79(4):203-6.

33. Aydin M, Hatirnaz O, Erensoy N, Ozbek U. CYP2D6 and CYP1A1 mutations in the Turkish population. Cell Biochem Funct 2005; 23 (2):133-5.