Hindawi
Genetics Research
Volume 2023, Article ID 8283470, 8 pages
https://doi.org/10.1155/2023/8283470

CAMBRIDGE
UNIVERSITY PRESS

Research Article

Allelic and Genotype Frequencies of CYP2B6*2 (64C > T) and CYP2B6*3 (777C > A) in Three Dominant Ethnicities of the Iranian Population

Armin Khavandegar,[1] **Bahareh Tavakoli-Far**,[2,3] **Sarina Ansari**,[1] **Parisa Veis-Karami**,[4] **Faezeh Ghasemi**,[4] **Samira Sheibaninia**,[4] **Roshanak Jazayeri**,[5] **and Massoud Houshmand**[4]

[1]*Student Research Committee, Alborz University of Medical Science, Karaj, Iran*
[2]*Dietary Supplements and Probiotic Research Center, Alborz University of Medical Sciences, Karaj, Iran*
[3]*Department of Physiology and Pharmacology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran*
[4]*Department of Medical Genetics, National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran*
[5]*Department of Genetics, Faculty of Medicine, Alborz University of Medical Sciences, Karaj, Iran*

Correspondence should be addressed to Roshanak Jazayeri; roshanakjazayeri@gmail.com and Massoud Houshmand; massoudh@nigeb.ac.ir

Received 29 December 2022; Revised 15 January 2023; Accepted 20 January 2023; Published 9 February 2023

Academic Editor: Chiara Mazziotta

Background. Cytochrome P450 complex plays a key role in drug metabolism. CYP2B6 has an essential part in Cytochrome P450 complex metabolism. This study aims to determine the allelic distribution of CYP2B6*2 and CYP2B6*3 in three main Iranian ethnicities: Fars, Turk, and Kurd. *Methods*. The study was conducted on 174 unrelated healthy volunteers from three main Iranian ethnicities. After DNA extraction from peripheral blood samples, genotyping of CYP2B6*2 and *3 was performed using tetra ARMS and ARMS PCR, respectively. *Results*. The average age of 174 cases was 40.69 ± 11.87 (mean ± SD) and 39.06 ± 11.63 (mean ± SD) for males and females. In the CYP2B6*2 variant, the genotyping frequency of wild type (C/C), heterozygous (C/T), and homozygous mutant (T/T) was 8.7%, 86%, and 5.2%, respectively. The CYP2B6*2 (c.64C > T) allele frequency was 48.2% (95% CI: (37.8–58.6)). In the CYP2B6*3 variant, the frequency of wild type (C/C), heterozygous (C/T), and homozygous mutant (T/T) was 75.3%, 11%, and 13.6%, respectively. The CYP2B6*3 (c.777C > A) allelic frequency was 19.1% (95% CI: (17.5–20.7)). *Conclusion*. Allelic distribution in three main Iranian ethnicities, i.e., Turk, Kurd, and Fars, is remarkably higher than that in other populations, even that in Southern Iran. High frequencies of CYP2B6*2 and *3 in the Iranian population highly affect drug responsiveness. Understanding such variability could help to increase drug efficacy and reduce its toxicity.

1. Introduction

Personalized medicine addresses the terms covered by targeted therapies, related diagnostics, and the medical intervention's safety [1]. Following the evolution of the Human Genome Project, the association between personalized medicine and genes encoding drug-metabolizing proteins became more outstanding [2]. Amongst all those proteins, Cytochrome P450, known as CYP, is responsible for the metabolization of roughly 90% of currently prescribed medications, apart from its intrinsic activities [3].

Cytochrome P450 enzymes play a crucial role in the metabolism of different agents, including food and drugs, with various enzymatic activities leading to various drug responses among individuals [4]. Many highly polymorphic genes encode cytochrome P450, and several

Single Nucleotide Polymorphisms (SNPs) have been identified in each gene [5]. SNPs may result in various enzymatic activities in people, eventually leading to insufficient efficacy of medical regimens or their adverse effects in a constant amount of drugs [6].

Ethnicity is among those factors influencing the SNP frequencies and various drug responses [7]. Although there are worldwide databases providing the frequency of multiple SNPs, they still need some data on specific ethnicities. Based on the latest studies, Iran's population was calculated at 83.9 million in 2020 and accounted for the 18th most populated country in the world [8]. Based on available data, 65% of the Iranian population are Fars, 16% Turk, 7% Kurd, 6% Lor, and 6% other ethnicities. In other words, Fars, Turk, and Kurd together account for 88% of the Iranian population [9].

CYP2B6 belongs to the Cytochromes P450 (CYPs) superfamily of enzymes that are essential for the clearance of various compounds, as well as for hormone synthesis [10, 11]. CYP2B6 participates in the metabolism of drugs, including many antibiotics, antiretroviral drugs, antimalarial, and first-lineanti-Tuberculosis drugs [12]. CYP2B6 is polymorphic and contains significant interindividual variability in the human population [13]. Hence, the analysis of CYP2B6 SNPs may improve drug efficacy and also decrease medical regimens' adverse effects.

Based on Lang et. al, CYP2B6 primarily consisted of 9 SNPs in 6 allelic variations; CYP2B6 c.64C > T as *2, CYP2B6 c.777C > A as *3, CYP2B6 c.785A > G as *4, CYP2B6 c.1459C > T as *5, CYP2B6 c.516G > T and c.785A > G as *6, and CYP2B6 c.516G > T and c.785A > G and c.1459C > T as *7 [14]. To date, the last CYP2B6 constellation is named *38 [15].

Studies on the CYP2B6 isozyme have expanded significantly since 2003, at the same time as the discovery of this isozyme effect in the clearance of the antiviral drug, especially efavirenz [13]. Besides, in another study conducted in 2003, the clearance of bupropion drug in people with the CYP2B6*4 variant was 1.6 times more than in patients of other variants [16]. A higher concentration of anti-HIV drugs, including efavirenz, and its toxicity in CYP2B6*2 [17] have been reported.

Various drugs are metabolized by CYP2B6 [18, 19]; among them, two drugs that are more specifically metabolized by a CYP2B6 isoenzyme are methadone and bupropion, and these two drugs are even utilized as probes to detect the function and investigate the behavior of the CYP2B6 enzyme [20, 21]. Reports show that Iran has a considerable rate of opium use, especially methadone [22]. To the extent of our knowledge, CYP2B6*2 and *3 have not been evaluated in Iranian populations, except for Southern Iran [23], which is previously reported to have a distinguished genetic pool from other Iranian populations [24].

In this study, due to the scarcity of data on the distribution of CYP2B6 c.64C > T (rs8192709) and CYP2B6 c.777C > A (rs45482602) in the Iranian population, we aimed to evaluate the mentioned polymorphism in three dominant Iranian ethnicities, Fars, Turk, and Kurd.

2. Methods and Materials

2.1. Samples Collection and Ethical Consideration. One hundred and seventy-four samples from unrelated healthy donors, aged 18–60, of three ethnicities of the Iranian population from various provinces, were achieved. Patients with a history of cancer, metabolic disorders, and any disease affecting DNA were excluded from the study. Of this number, 65 (37.4%) cases were males and 109 (62.6%) were females. The average age of cases was 40.69 ± 11.87 (mean ± SD) and 39.06 ± 11.63 (mean ± SD) for males and females, respectively. Two milliliters of blood samples encompassing 80 Fars, 69 Turk, and 25 Kurd were collected in ethylenediaminetetraacetic acid (EDTA) enriched tubes. An informed written consent form was obtained from each person to permit genetic analysis and publication. Besides, the Medical ethics committee has approved the study.

2.2. DNA Extraction and Primer Sequencing. DNA extraction was performed using an MBST kit (salting-out method) in two milliliters of blood samples. For DNA isolation, lysis buffer for the digestion of non-nucleic acid components of the cell, precipitation buffer for protein isolation, washing buffer, and eventually elusion buffer for DNA resuspension were utilized. Therefore, we utilized Oligonucleotide F (forward) and R (reverse) primers for CYP2B6 *2 and *3 amplification, as shown in Table 1.

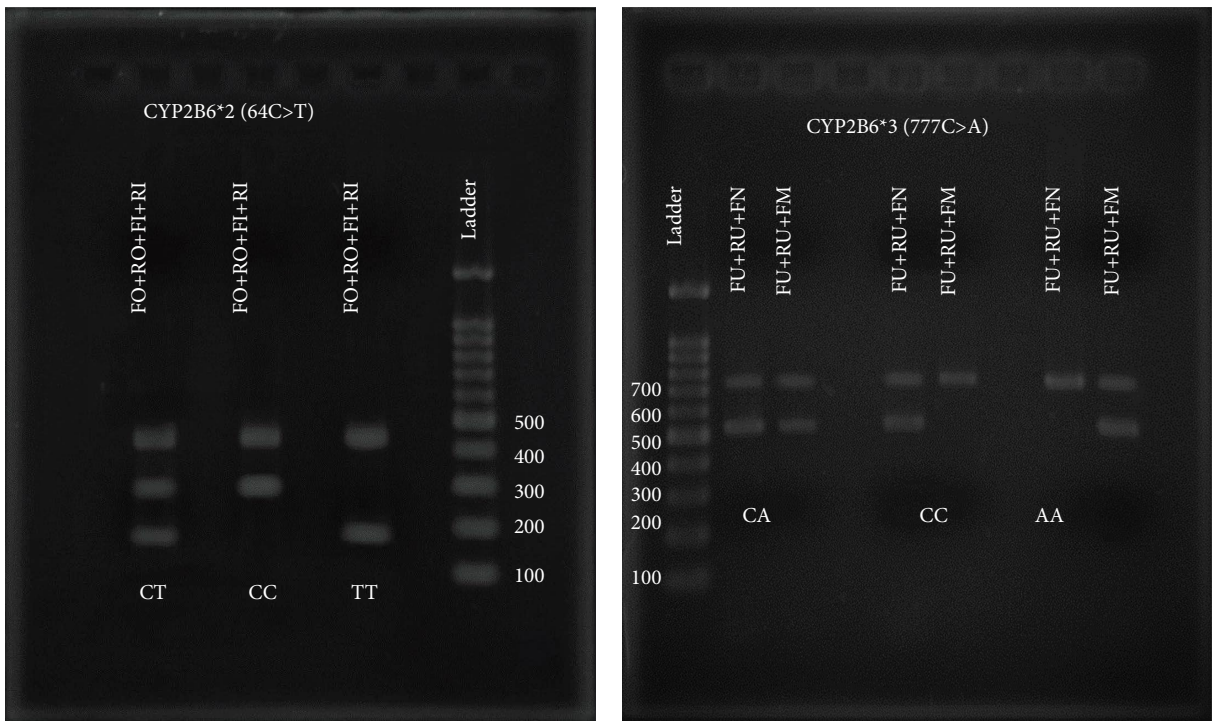
2.3. ARMS and Tetra ARMS-PCR. Genotyping analysis was conducted utilizing an amplification refractory mutation system polymerase chain reaction (ARMS PCR) for CYP2B6*3 and tetra ARMS PCR for CYP2B6*2. The total volume of each PCR reaction was 25 microliters consisting of 0.6 μl of each F and R primers, two μl DNA template, and 11 μl EmeraldAmp PCR master mix. The PCR cycling was set at 96°C for 4 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing for 45 seconds, extension at 72°C for 45 seconds, and final extension at 72°C for 8 minutes. The 3% agarose gel was used to rub the PCR products. Eventually, visualization was performed by staining with ethidium bromide and analyzed by the gel documentation system (Figure 1).

2.4. Sequencing Analysis. Similar to our previous studies [25], forwards and reverse primers sequenced PCR products on an automated ABI 3100 sequencing machine (Applied Biosystems, Kavosh Fanavaran Kawsar Company, Iran). Then, we used the Finch TV program for sequencing and analyzing to confirm the results of nucleotide variations.

2.5. Statistical Analysis. The statistical analyses were performed using SPSS (version 24; IBM) software. A confidence interval test (95%) was considered for the frequency of alleles and genotypes. A P value of less than 0.05 was considered statistically significant [25].

Table 1: Oligonucleotide sequences of primers in real-time, rapid-cycle PCR.

SNP	Primer	Sequence	Position	PCR technique
CYP2B6 c.64C > T (p.R22 > C) or rs8192709 or*2	FO	5′GGGATAGGCATCAGGTCACTGG3′	FO-RO 431	Tetra ARMS
	RO	5′TTCCCCAAGTACCAAGGCAAGA3′	FO-RI 283	
	FI	5′CTCTTGCTACTCCTGGTTCAGT3′	FI-RO 189	
	RI	5′TCATGGGTGTTAGGGTGGCG3′		
CYP2B6 c.777C > A (p.S259 > R) or rs45482602 or*3	FU	5′GTTCCCATGGAGGGATTGGG3′	FU-RU 734	ARMS
	RU	5′CTCTACACATCCAACCGCGTA3′	FN-RU 525	
	FM	5′ GAAACCCTGGACCCCAGA 3′	FM-RU 525	
	FN	5′ GAAACCCTGGACCCCAGC3′		



(a) (b)

Figure 1: (a) A heterozygous, wild type, and homozygous mutant, left to right, in rs8192709 in three cases after tetra ARMS-PCR. (b) A heterozygous, wild type, and homozygous mutant, left to right, in rs45482602 in three cases after ARMS-PCR.

Table 2: Analysis of the CYP2B6*2 allelic frequencies (%) among three dominant ethnicities in Iran; the correlation between ethnicity genotype frequency ethnicity and the each ethnicity is shown.

Number (%)		Ethnicity			
		Fars 80 (46%)	Turk 69 (40%)	Kurd 25 (14%)	Total 174 (100%)
CYP2B6*2	Allele C% (95% CI)	52 (47.8–56.2)	50 (47.4–52.6)	56 (49.2–62.2)	51.8 (47.1–56.5)
	Allele T% (95% CI)	48 (44.6–51.4)	50 (41.6–58.4)	44 (36.8–51.2)	48.2 (37.8–58.6)
	P value	0.179	0.401	0.386	
CYP2B6*3	Allele C% (95% CI)	79.4 (71.6–87.2)	83.4 (70–96.8)	80 (74.4–85.6)	80.9 (74.1–87.7)
	Allele A% (95% CI)	20.6 (16.2–25)	16.6 (12.8–20.4)	20 (17.8–22.2)	19.1 (17.5–20.7)
	P value	0.609	0.796	0.681	

3. Results

3.1. Demographic Distribution. In this study, 174 samples of whole blood cells were collected from three dominant Iranian ethnicities consisting of 80 (46%) Fars, 69 (39.7%) Turk, and 25 (14.4%) Kurd to investigate the allelic and phenotypic distribution of CYP2B6*2 and *3. There was no significant correlation between the samples' gender and allelic genotypes.

3.2. CYP2B6 (c.64 > T) Allelic and Genotype Frequency. CYP2B6 wild-type homozygote (C/C) was 87% (95% CI (6.7–10.7)), while mutated homozygote (T/T) and heterozygote (C/T) genotypes were 5.2% (95% CI (3.9–6.5)) and 86% (95% CI (83.2–88.8)), respectively. C/C genotype highest and lowest frequencies were observed in Kurd (12% (95% CI (10.3–13.7))) and Fars (6.3% (95% CI (5.7–6.9))), respectively. T/T genotype was highest in Turk (10.3% (95% CI (5.2–15.4))) and lowest in Kurd (0%). Furthermore, C/T genotype ranged from 91.1% (95% CI (87.5–94.7)) in Fars to 79.4% (95% CI (73.5–85.5)) in Turk. The CYP2B6*2 (c.64 C > T) allele was 48.2% (95% CI (37.8–58.6)), ranging from 44% (95% CI (36.8–51.2)) in Kurd to 50% (95% CI (41.6–58.4)) in Turk. Tables 2 and 3 summarize the genotype and allelic frequency, respectively.

*3.3. CYP2B6*3 (c.777C > A) Allelic and Genotype Frequency.* CYP2B6 wild-type homozygote (C/C) was 75.3% (95% CI (70.1–80.5)), while mutated homozygote (A/A) and heterozygote (C/A) genotypes were 13.6% (95% CI

Table 3: Analysis of the CYP2B6*2 genotypic frequencies (%) among three dominant ethnicities in Iran; the correlation between ethnicity genotype frequency ethnicity and the each ethnicity is shown.

	Number (%)	Ethnicity			
		Fars 80 (46%)	Turk 69 (40%)	Kurd 25 (14%)	Total 174 (100%)
CYP2B6*2	Wild (C/C) (95% CI)	6.3% (5.7–6.9)	10.3% (9.3–11.3)	12% (10.3–13.7)	8.7% (6.7–10.7)
	Heterozygous (C/T) (95% CI)	91.1% (87.5–94.7)	79.4% (73.5–85.5)	88% (80.2–95.8)	86% (83.2–88.8)
	Mutant (T/T) (95% CI)	2.5% (1.7–3.3)	10.3% (5.2–15.4)	0%	5.2% (3.9–6.5)
	P value	<0.001**	0.041*	<0.001**	
CYP2B6*3	Wild (C/C) (95% CI)	74.7% (70.6–78.8)	77.8% (74.6–81)	72% (65.3–78.7)	75.3% (70.1–80.5)
	Heterozygous (C/A) (95% CI)	9.3% (7.2–11.4)	11.1% (6.6–15.6)	16% (11.8–20.2)	11% (8.3–13.7)
	Mutant (A/A) (95% CI)	16% (13.7–18.3)	11.1% (7.4–14.8)	12% (9.1–14.9)	13.6% (12.5–14.7)
	P value	<0.001**	<0.001**	<0.001**	

*P value of less than 0.05 is statistically significant. **P value of less than 0.01 is statistically highly significant.

Table 4: Comparison of allele frequency of CYP2B6*2 reported in various populations.

Authors	Year	Population	*2 frequency (%)	Heterozygous Homozygous	Sample size	Reference
Current study	2022	Iranian	48.2	86% 5.2	174	
Thomas Lang	2001	Caucasian	5.4	10.7% 0	215	[14]
Hiratsuka et al.	2002	Japanese	4.7	N/A N/A	530	[31]
Hiratsuka et al.	2002	Caucasian	5.3	N/A N/A	430	[31]
Mehlotra et al.	2006	West Africa	2.82	N/A N/A	631	[32]
Musa et al.	2012	Malaysia	0.8	N/A N/A	392	[33]
Musa et al.	2012	Chinese	1.3	N/A N/A	330	[33]
Musa et al.	2012	Indians	4.1	N/A N/A	126	[33]
Zakeri et al.	2014	Southern Iran	3.9	N/A N/A	206	[23]
Tomas et al.	2017	Croatian Roma	12.8	22.9% 1.4%	436	[35]
Cho et al.	2004	Korean	3	N/A N/A	358	[36]
Lamba et al.	2003	Hispanics	14	N/A N/A	14	[37]
Lamba et al.	2003	Caucasians	9	N/A N/A	86	[37]
Carano et al.	2018	Italian	6.61	N/A N/A	348	[38]
Arnaldo et al.	2013	Mozambican	5.7	7.8% 1.9%	360	[39]

(12.5–14.7)) and 11% (95% CI (8.3–13.7)), respectively. C/C genotype highest and lowest frequencies were observed in Turk (77.8% (95% CI (74.6–81))) and Kurd (72% (95% CI (65.3–78.7))), respectively. A/A genotype was highest in Fars (16% (95% CI (13.7–18.3))) and lowest in Turk (11.1% (955 CI (7.4–14.8))). Furthermore, C/A genotype ranged from 9.3% (95% CI (7.2–11.4)) in Fars to 16% (95% CI (11.8–20.2)) in Kurd. The CYP2B6*3 (c.777C > A) allele was 19.1% (95% CI (17.5–20.7)), ranging from 16.6% (95% CI(12.8–20.4)) in Turk to 20.6 (95% CI (16.2–25)) in Fars. Tables 2 and 3 summarize the genotype and allelic frequency, respectively.

Table 5: Comparison of allele frequency of CYP2B6*3 reported in various populations.

Authors	year	Sample size	*3 frequency (%)	Heterozygous Homozygous	Sample size	Reference
Current study	2022	Iranian	19.1	11% 13.6%	174	
Thomas Lang	2001	Caucasian	0.5	0.9% 0	215	[14]
Hiratsuka et al.	2002	Japanese	0	N/A N/A	530	[31]
Hiratsuka et al.	2002	Caucasian	0.5	N/A N/A	430	[31]
Mehlotra et al.	2006	West Africa	0	N/A N/A	631	[32]
Ahmed et al.	2021	Pakistan	6.5	6.73% 3.06%	490	[34]
Cho et al.	2004	Korean	0	0 0	358	[36]
Arnaldo et al.	2013	Mozambican	0	0 0	360	[39]

4. Discussion

CYP2B6 plays a crucial role in the metabolism of antiretroviral drugs, including efavirenz, immunosuppressant drugs, including cyclophosphamide, antidepressants, e.g., bupropion, antiepileptics, e.g., valproic acid, and antiarrhythmic, e.g., mexiletine, among other drugs, by changing to their metabolic forms [13, 26–29]. Also, it is believed that CYP2B6 expression is higher in smokers and alcoholics compared to the normal population [30].

To the extent of our knowledge, this is the first study investigating the frequency of CYP2B6*2 and CYP2B6*3 in the three main Iranian populations: Fars, Turk, and Kurd. In this study, CYP2B6*2 frequency was 48.2%, ranging from 44% in Kurd to 50% in Turk ethnicities; besides, CYP2B6*3 frequency was 19.1% on average, ranging from 16.6% in Turk to 20% in Kurd. In a survey in Japan by Hiratsuka et al., CYP2B6*2 and CYP2B6*3 frequencies were 4.7% and 0%, respectively; besides, the same alleles frequencies in the Caucasian population were reported at 5.3% and 0.5%, respectively [31].

In another study evaluating the CYP2B6 allelic distribution in 631 West African populations, the frequency of CYP2B6*3 was 0%, while the frequency of CYP2B6*2 was 2.82% [32]. In a study by Musa et al., CYP2B6*2 allelic distribution was 0.8% in Malaysian and 4.1% in Indians [33]. Another study investigating the allelic frequency of CYP2B6 in ethnicity in Southern Iran, known as the Baluch population, reported a 3.9% allelic distribution of CYP2B6*2 [23]. In a study in 2021, CYP2B6*3 allelic distribution was reported at 6.5% of the Pakistan population [34].

As demonstrated in Tables 4 and 5, the CYP2B6*2 and *3 allelic frequency is remarkably higher in the three main Iranian populations than in other ethnicities, even those in Southern Iran. It is believed that due to a considerable number of consanguineous marriages in Iran, autosomal recessive diseases highly manifest in this country; furthermore, it is said that Iranian allele frequency is noticeably different from those in Europeans, while Iranian Baluch seemed to have a tendency towards south Asians in allele frequency [23, 24]. Besides, CYP2B is believed to have the most frequent interpersonal variety, which accounted for 20–250 times more than other Cytochrome P450 isoenzymes [40].

Our results, alongside those from other studies, provide further evidence for ethnic heterogeneity in drug metabolism. We hope our findings contribute to a better understanding of various drug responses in different populations.

5. Study Limitations

This study was conducted on 173 cases from three various major Iranian ethnicities. Further studies are needed to evaluate the allelic polymorphism in larger populations and other ethnicities.

6. Conclusion

Allelic distribution in three main Iranian ethnicities, i.e., Turk, Kurd, and Fars, is remarkably different from other populations, even those in Southern Iran. High frequencies of CYP2B6*2 and *3 in the Iranian population remarkably affect drug responsiveness, including higher efavirenz toxicity. Understanding such variability could help to increase drug efficacy and reduce its toxicity.

Data Availability

We have presented all the necessary data in this manuscript. The data would be available from the corresponding author on reasonable request.

Ethical Approval

This study is approved by the Ethics Board Committee of Alborz University of Medical Sciences with the approval ID: IR.ABZUMS.REC.1397.169.

Consent

Informed consent forms were obtained from all participants for participation in the study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

A.Kh contributed to the study design and methodology. R.J. and M.H. supervised the whole process and modified it. B.T. contributed to manuscript provision, data analysis, and submission process. S.A. and P.V. contributed to writing the original draft. F.Gh and S.Sh helped in study coordination. All authors have read and approved the manuscript.

Acknowledgments

The authors are grateful to all residents who participated in this study.

References

[1] F. De Andrés, S. Teran, F. Hernandez, E. Teran, and A. Llerena, "To genotype or phenotype for personalized medicine? CYP450 drug metabolizing enzyme genotype–phenotype concordance and discordance in the Ecuadorian population," *OMICS: A Journal of Integrative Biology*, vol. 20, no. 12, pp. 699–710, 2016.

[2] Q. Chen and D. Wei, "Human cytochrome P450 and personalized medicine," *Advances in Experimental Medicine and Biology*, vol. 827, pp. 341–351, 2015.

[3] Q. Chen, T. Zhang, J. F. Wang, and D. Q. Wei, "Advances in human cytochrome p450 and personalized medicine," *Current Drug Metabolism*, vol. 12, no. 5, pp. 436–444, 2011.

[4] S. I. Hamdy, M. Hiratsuka, K. Narahara et al., "Allele and genotype frequencies of polymorphic cytochromes P450 (CYP2C9, CYP2C19, CYP2E1) and dihydropyrimidine dehydrogenase (DPYD) in the Egyptian population," *British Journal of Clinical Pharmacology*, vol. 53, no. 6, pp. 596–603, 2002.

[5] S.-F. Zhou, "Polymorphism of human cytochrome P450 2D6 and its clinical significance," *Clinical Pharmacokinetics*, vol. 48, no. 12, pp. 761–804, 2009.

[6] V. M. Lauschke and M. Ingelman-Sundberg, "The importance of patient-specific factors for hepatic drug response and toxicity," *International Journal of Molecular Sciences*, vol. 17, no. 10, p. 1714, 2016.

[7] A. Helgadottir, A. Manolescu, A. Helgason et al., "A variant of the gene encoding leukotriene A4 hydrolase confers ethnicity-specific risk of myocardial infarction," *Nature Genetics*, vol. 38, no. 1, pp. 68–74, 2006.

[8] Worldometers, "Statistical center of Iranian Population," 2020, https://www.worldometers.info/world-population/iran-population/.

[9] Amar, "Statistical center of Iran," 2011, https://www.amar.org.ir/english.

[10] J. C. Rotondo, L. Giari, C. Guerranti et al., "Environmental doses of perfluorooctanoic acid change the expression of genes in target tissues of common carp," *Environmental Toxicology and Chemistry*, vol. 37, no. 3, pp. 942–948, 2018.

[11] A. M. McDonnell and C. H. Dang, "Basic review of the cytochrome p450 system," *Journal of the Advanced Practitioner in Oncology*, vol. 4, no. 4, pp. 263–268, 2013.

[12] U. S. Simonsson, B. Jansson, and T. N. Hai, "Artemisinin autoinduction is caused by involvement of cytochrome P450 2B6 but not 2C9," *Clinical Pharmacology and Therapeutics (St. Louis)*, vol. 74, no. 1, pp. 32–43, 2003.

[13] B. A. Ward, J. C. Gorski, D. R. Jones, S. D. Hall, D. A. Flockhart, and Z. Desta, "The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity," *Journal of Pharmacology and Experimental Therapeutics*, vol. 306, no. 1, pp. 287–300, 2003.

[14] T. Lang, K. Klein, J. Fischer et al., "Extensive genetic polymorphism in the human CYP2B6 gene with impact on expression and function in human liver," *Pharmacogenetics*, vol. 11, no. 5, pp. 399–415, 2001.

[15] A. Anagnostopoulos, M. Rotger, M. Aouri et al., "Efavirenz intoxication due to a new CYP2B6 constellation," *Antiviral Therapy*, vol. 18, no. 5, pp. 739–743, 2013.

[16] J. Kirchheiner, C. Klein, I. Meineke et al., "Bupropion and 4-OH-bupropion pharmacokinetics in relation to genetic polymorphisms in CYP2B6," *Pharmacogenetics*, vol. 13, no. 10, pp. 619–626, 2003.

[17] O. Usami, Y. Ashino, Y. Komaki et al., "Efavirenz-induced neurological symptoms in rare homozygote CYP2B6* 2/* 2 (C64T)," *International Journal of STD & AIDS*, vol. 18, no. 8, pp. 575-576, 2007.

[18] H. Yamada, Y. Ishii, M. Yamamoto, and K. Oguri, "Induction of the hepatic cytochrome P450 2B subfamily by xenobiotics: research history, evolutionary aspect, relation to tumorigenesis, and mechanism," *Current Drug Metabolism*, vol. 7, no. 4, pp. 397–409, 2006.

[19] E. Hodgson and R. L. Rose, "The importance of cytochrome P450 2B6 in the human metabolism of environmental chemicals," *Pharmacology & Therapeutics*, vol. 113, no. 2, pp. 420–428, 2007.

[20] E. D. Kharasch, D. Mitchell, and R. Coles, "Stereoselective bupropion hydroxylation as an in vivo phenotypic probe for cytochrome P4502B6 (CYP2B6) activity," *The Journal of Clinical Pharmacology*, vol. 48, no. 4, pp. 464–474, 2008.

[21] C. B. Eap, S. Crettol, J. S. Rougier et al., "Stereoselective block of hERG channel by (S)-methadone and QT interval prolongation in CYP2B6 slow metabolizers," *Clinical Pharmacology and Therapeutics*, vol. 81, no. 5, pp. 719–728, 2007.

[22] Y. Rostam-Abadi, J. Gholami, A. Noroozi et al., "Public health risks associated with methadone in Iran: a systematic review and meta-analysis," *International Journal of Drug Policy*, vol. 100, Article ID 103529, 2022.

[23] S. Zakeri, N. Amiri, S. Pirahmadi, and N. Dinparast Djadid, "Genetic variability of CYP2B6 polymorphisms in southeast Iranian population: implications for malaria and HIV/AIDS treatment," *Archives of Iranian Medicine*, vol. 17, no. 10, pp. 685–691, 2014.

[24] Z. Mehrjoo, Z. Fattahi, M. Beheshtian et al., "Distinct genetic variation and heterogeneity of the Iranian population," *PLoS Genetics*, vol. 15, no. 9, Article ID e1008385, 2019.

[25] M. Dehbozorgi, B. Kamalidehghan, I. Hosseini et al., "Prevalence of the CYP2C19* 2 (681 G> A),* 3 (636 G> A) and* 17 (-806 C> T) alleles among an Iranian population of different ethnicities," *Molecular Medicine Reports*, vol. 17, no. 3, pp. 4195–4202, 2018.

[26] T. K. Chang, G. F. Weber, C. L. Crespi, and D. J. Waxman, "Differential activation of cyclophosphamide and ifosphamide by cytochromes P-450 2B and 3A in human liver microsomes," *Cancer Research*, vol. 53, no. 23, pp. 5629–5637, 1993.

[27] L. M. Hesse, K. Venkatakrishnan, M. H. Court et al., "CYP2B6 mediates the in vitro hydroxylation of bupropion: potential drug interactions with other antidepressants," *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, vol. 28, no. 10, pp. 1176–1183, 2000.

[28] T. K. L. Kiang, P. C. Ho, M. R. Anari, V. Tong, F. S. Abbott, and T. K. H. Chang, "Contribution of CYP2C9, CYP2A6, and CYP2B6 to valproic acid metabolism in hepatic microsomes from individuals with the CYP2C9* 1/* 1 genotype," *Toxicological Sciences*, vol. 94, no. 2, pp. 261–271, 2006.

[29] L. Labbe, Z. Abolfathi, E. Lessard, H. Pakdel, P. Beaune, and J. Turgeon, "Role of specific cytochrome P450 enzymes in the N-oxidation of the antiarrhythmic agent mexiletine," *Xenobiotica*, vol. 33, no. 1, pp. 13–25, 2003.

[30] S. Miksys, C. Lerman, P. G. Shields, and D. C. Mash, "Smoking, alcoholism and genetic polymorphisms alter CYP2B6 levels in human brain," *Neuropharmacology*, vol. 45, no. 1, pp. 122–132, 2003.

[31] M. Hiratsuka, Y. Takekuma, N. Endo et al., "Allele and genotype frequencies of CYP2B6 and CYP3A5 in the Japanese population," *European Journal of Clinical Pharmacology*, vol. 58, no. 6, pp. 417–421, 2002.

[32] R. K. Mehlotra, M. N. Ziats, M. J. Bockarie, and P. A. Zimmerman, "Prevalence of CYP2B6 alleles in malaria-endemic populations of West Africa and Papua New Guinea," *European Journal of Clinical Pharmacology*, vol. 62, no. 4, pp. 267–275, 2006.

[33] N. Musa, N. Musa, N. Talib, N. Mohamad, and M. Zulkafli, "Haplotypes frequencies of CYP2B6 in Malaysia," *Journal of Postgraduate Medicine*, vol. 58, no. 4, p. 235, 2012.

[34] S. Ahmed, S. Khan, K. Janjua, I. Imran, and A. U. Khan, "Allelic and genotype frequencies of major CYP2B6 polymorphisms in the Pakistani population," *Molecular Genetics & Genomic Medicine*, vol. 9, no. 3, Article ID e1527, 2021.

[35] Ž. Tomas, A. Kuhanec, T. Skaric-Juric et al., "Distinctiveness of the Roma population within CYP2B6 worldwide variation," *Pharmacogenomics*, vol. 18, no. 17, pp. 1575–1587, 2017.

[36] J.-Y. Cho, H. S. Lim, J. Y. Chung et al., "Haplotype structure and allele frequencies of CYP2B6 in a Korean population," *Drug Metabolism and Disposition*, vol. 32, no. 12, pp. 1341–1344, 2004.

[37] V. Lamba, J. Lamba, K. Yasuda et al., "Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression," *Journal of Pharmacology and Experimental Therapeutics*, vol. 307, no. 3, pp. 906–922, 2003.

[38] F. Carano, S. Sarno, S. De Fanti et al., "Genetic variability of CYP2D6, CYP2B6, CYP2C9 and CYP2C19 genes across the Italian Peninsula," *Annals of Human Biology*, vol. 45, no. 1, pp. 66–71, 2018.

[39] P. Arnaldo, R. E. Thompson, M. Q. Lopes, and P. N. Suffys, *Frequencies of Cytochrome P450 2B6 and 2C8 Allelic Variants in the Mozambican Population*, Penerbit Universiti Sains, Penerbit, Malasiya, 2013.

[40] H. Wang and L. M. Tompkins, "CYP2B6: new insights into a historically overlooked cytochrome P450 isozyme," *Current Drug Metabolism*, vol. 9, no. 7, pp. 598–610, 2008.