British Journal of Medicine & Medical Research
4(10): 2081-2088, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Genetic Polymorphisms of CYP2C8 in A Healthy Iranian Population

Ali Ramazani[1*], Mahsa Ghaffari[1] and Amir Fakhri[2]

[1]Zanjan Pharmaceutical Biotechnology Research Center, Zanjan University of Medical Sciences, Zanjan, Iran.
[2]Department of Pharmacology and Toxicology, School of Pharmacy, Shahid Beheshti University of Medical Science, Tehran, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. Author AR directed the implementation of the study and data analysis. Author MG carried out the experiments and drafted the manuscript. Author AF made statistical analysis. All authors read and approved the final manuscript.

Original Research Article

Received 22nd October 2013
Accepted 27th December 2013
Published 18th January 2014

ABSTRACT

Aim: The aims of this study were to genotype *CYP2C8* in an Iranian population and compare their allelic frequencies with other ethnic groups.
Study Site and Duration: Biotechnology Department, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran from June 2012 through May 2013.
Methodology: *CYP2C8* (*1/*2/*3) allelic variants were determined in 200 unrelated healthy Iranian volunteers by real time-polymerase chain reaction (PCR).
Results: A total 156 subjects (78%) were homozygous for *CYP2C8*1*, six subjects (3%) were homozygous for *CYP2C8*2* and 38 subjects (19%) were heterozygous *CYP2C8*1/*2*. *CYP2C8*3* was not detected.
Discussion and Conclusion: Genotyping indicated no significant (P>0.05) difference between *CYP2C8* allelic variant frequencies in an Iranian compared with Burkina Faso population. The Iranian population's *CYP2C8* allelic variation was significantly (P<0.05) different when compared with populations in Portugal, African-American race to Malaysia, Ghana, Zanzibar, Spain, Czech Republic and Sweden.

**Corresponding author: Email: ramazania@zums.ac.ir;*

Keywords: CYP2C8; real-time PCR; allele frequency; Iran.

1. INTRODUCTION

Cytochrome P450s (CYPs) are a group of microsomal heme-thiolate monooxygenases that are involved in an NADPH-dependent electron transport pathway. CYPs also oxidize a variety of structurally unrelated compounds including steroids, fatty acids, and xenobiotics. CYPs also said the metabolism and elimination of a wide range of medications as well as other xenobiotics. CYPs mediate the biotransformation of lipophilic compounds into polar metabolites that are eliminated by urinary or biliary excretion [1]. The human hepatic CYP system consists of over 30 related isoenzymes. Genetic polymorphisms in the CYP2C8 isoform cause variability in drug response. Specifically, genetic variation in a gene encoding a drug-metabolizing enzyme leads to high, low, or no enzyme activity [2]. Therefore, populations are divided into poor metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM) and ultra-rapid metabolizer (UM) phenotypes [3-4].

The human CYP2C subfamily consists of four known isoforms (2C8, 2C9, 2C18 and 2C19) that have closely linked genic loci located on chromosome 10 [5]. CYP2C enzymes account for ~20% of all microsomal drug metabolizing CYPs [6-7]. CYP2C8 is the principal enzyme that metabolizes the anti-cancer drug paclitaxel (taxol), the antimalarial drug amodiaquine, the hypoglycaemic drug troglitazone, amiodarone, verapamil, and ibuprofen. CYP2C8 also metabolizes fluvastatin, amitriptyline, perphenazine, diclofenac, gallopamil, omeprazole, and carbamazepine [8-9]. At least 16 different *CYP2C8* alleles have been reported [10]. Four nonsynonymous variant alleles designated as *CYP2C8*2-*5* (wild-type, *CYP2C8*1*) decrease enzymatic activity [11].

CYP2C8*2 is widespread [13] with an allele frequency of 0.18 in African-American populations, whereas *CYP2C8*3* and *CYP2C8*4* (allele frequencies of 0.13 and 0.075, respectively) are found primarily in Caucasian populations [12]. This allele occurs at different frequencies in West and East African populations [13]. Similar to Caucasians, the *CYP2C8*2* is virtually absent in other non-African populations [11]. *CYP2C8*3*, an allele with the PM phenotype is absent or found at very low frequencies in Africans [11].

There are significant ethnic differences in the distribution of *CYP2C8* alleles [15-16]. Nearly 10%–20% of Caucasians carry the *CYP2C8*3* variant, whereas this allele is rare in African Americans [14]. Patients homozygous for *CYP2C8*2* or *CYP2C8*3* have lower intrinsic clearance of CYP2C8 substrates than *CYP2C8*1/*2* or *CYP2C8*1/*3* heterozygote's [13,17-18]. *CYP2C8*2* results from a single base pair trans version (805A>T) that creates an aberrant splice site resulting in an enzyme with low activity. *CYP2C8*3* results from a transition (416G>A) and creates a weak enzyme and PM phenotype [11,19].

The two main *CYP2C8* (*2 and *3) variants identified in previously genotyped populations were determined in 200 unrelated healthy Iranian volunteers by real time-PCR. Real time-PCR was used because it is highly sensitive, specific and capable of measuring low copy number DNA samples. This study is done for the first time in Iranian population and the result can be doted to Iranian population.

2. MATERIALS AND METHODS

2.1 Sample Collection

The blood samples were taken from two hundred unrelated healthy Iranian volunteers, 92 (46%) males and 108 (54%) females, aged between 19-57 years, mean age 28.72 from different Provinces of Iran between 2011 to 2012 years. The ethnicity of voluntaries was as follow: Persians (Tehran and Fars), Kurds (Kermanshah and Kurdistan), Gilakis (Gilan), Turks (Zanjan and Azerbaijan), Tats (Qazvin) and Lurs (Lorestan) Fig. 1. The study was done at Zanjan University of Medical Sciences, Zanjan, Iran during June, 2012 to May, 2013. The study protocol was approved by the ethics committee of the Zanjan University of Medical Sciences and written informed consent to participate in the study was obtained from the volunteers.

Five ml of venous blood was obtained from each subject. DNA was manually extracted from peripheral blood leukocytes by salting-out method [20]. Blood cell lyses buffer containing SDS (Sodium Dodecyl Sulfate), proteinase K, and sodium chloride (NaCl) was used for DNA extraction. DNA samples were concentrated by Ethanol (70%), recovered by centrifugation and re-suspended in TE buffer (Tris-HCl EDTA pH=8.8). Concentration and purity of the DNA was determined spectrophotometrically (BioPhotometer plus, Eppendorf, Germany) by reading absorbance at 260 and 280 (A260 and A280). DNA samples were stored at 4ºC.

Fig. 1. Map of Iran on which the residence of volunteers (■) were indicated

2.2 Genotyping of the *CYP2C8* Variant Alleles

TaqMan® conventional probes and primers were designed by Primer Express® Software v3.0.1 (Applied Biosystems, USA) and synthesized by Bioneer Company (South Korea). Dual-labeled, TaqMan® probes labeled with both a fluorophore and a quencher dye are used in real-time PCR assays to detect amplification of specific SNP targets. The fluorophore FAM (6-carboxyfluorescein) was used at 5' end of wild type probes and HEX (hexachloro-6-carboxyfluorescein) for variant one. The fluorophore TAMRA (tetramethylrhodamine) was used as quencher dye at 3' end of both wild type and variant probes. Allelic discrimination was performed using TaqMan® SNP Genotyping Assays on the Rotor-Gene 6000 (Corbett, Australia).

Real-time PCR was performed in a 20 µL reaction mixtures containing 10 µL qPCR prob Master Mix (Jena Bioscience, Germany), 20 nM of each probe (wild type and variant allele), 10 pM of each specific forward and reverse primers and 5 ng of extracted DNA. These concentrations were applied for two allelic variants. The amplification condition was as follows: First, initial heat activation at 95ºC for 10 min, followed by 45 cycles of 95ºC for 10s and 60ºC for 1 min. For allelic variants genotyping quality control, 5% of samples were genotyped in duplicate and confirmed by sequencing (Bioneer, South Korea). The nucleotide sequence of all PCR primers and probes are listed in Table 1.

Differences in allele frequency between different populations were determined using chi square test. Deviations from Hardy Weinberg Equilibrium were also assessed using a chi square test. All analyses were performed using SPSS 16.0 for windows.

Table 1. The nucleotide sequence of all PCR primers and probes for CYP2C8*2 and CYP2C8*3 detection

Allele	Oligo Name	Sequence (5'-3')	Tm	%GC	Amplicon (bp)
*CYP2C8*2*	Forward Primer	GGATGTTAACAATCCTCGGGAC	58.3	50	110
	Reverse Primer	ATGAATCACAAAATGGACAAGAAATC	58.4	31	
	Probe (Wild type)	FAM-ATCGATTGCTTCCTGATCAAAATGGAG-TAMRA	65.1	41	
	Probe (Variant)	HEX-TCGATTGCTTCCTGTTCAAAATGGAG-TAMRA	65.1	42	
*CYP2C8*3*	Forward Primer	CAATGGAAAGAGATGGAAGGAGAT	58.6	42	110
	Reverse Primer	GGCAGTGAGCTTCCTCTTGAA	58.3	52	
	Probe (Wild type)	FAM-CGGTCCTCAATGCTCCTCTTCCC-TAMRA	66.0	61	
	Probe (Variant)	HEX-TCCTCAATGCTCTTCTTCCCCATCC-TAMRA	66.3	52	

3. RESULTS

Iranian *CYP2C8* allelic variation and genotypic frequencies are summarized in Table 2. *CYP2C8*1, CYP2C8*2* and *CYP2C8*3* allele frequencies were 82%, 18%, and 0% respectively. There were 156 subjects (78%) with the *CYP2C8*1/*1* genotype (wild-type), 38 subjects (19%) with the *CYP2C8*1/*2* genotype, and six subjects (3%) with the *CYP2C8*2/*2* genotype. No significant (P>0.05) difference in allele frequencies between males and females was detected. *CYP2C8* allele frequencies were consistent with Hardy Weinberg equilibrium (P value≥0.05) among the studied population (χ^2 = 6.9093, P = 0.2).

Table 2. Allele and Genotype frequencies of *CYP2C8* among Iranian volunteers

Alleles/Genotype	Number	Frequency
*CYP2C8*1*	164	0.82
*CYP2C8*2*	36	0.18
*CYP2C8*3*	0	0
**1/*1*	156	0.78
**1/*2*	38	0.19
**2/*2*	6	0.03
**1/*3*	0	0
**3/*3*	0	0
**2/*3*	0	0

4. DISCUSSION

Advances in medical and human genetics have enabled a more detailed understanding of genetics in disease. Furthermore, large collaborative research projects (such as, the human genome project) have established a foundation for understanding gene function with respect to human development and physiology, revealed single nucleotide polymorphisms (SNPs) that underlie inter-individual genetic variability, and made genome-wide association studies (GWAS) that are used to examine genetic variation and risk for many common diseases possible [21].

The discovery of functional variability between drug metabolizing enzymes has significantly contributed to understanding inter-individual variability in dose -concentration and -response relationships. Polymorphism of *CYP2C8* has to date been less well characterized than other CYP families. *CYP2C8*3* allele was not detected in our population. Our results demonstrated that the *CYP2C8*2* allele is widespread in Iran and suggest that some Iranians may have decreased CYP2C8 activity and a PM phenotype. We also identified similarities (P>0.05) between *CYP2C8* allelic variation and genotypic distribution patterns in Iranians and Burkina Faso populations [22] Table 3. The Iranian population's *CYP2C8* allelic variation was significantly (P<0.05) different when compared with populations in Portugal, African-American race to Malaysia, Ghana, Zanzibar, Spain, Czech Republic, Sweden Table 3.

Table 3. Distribution of *CYP2C8* variant alleles among different ethnic groups

Population	Study	Allele frequency		*P* value
		CYP2C8 *2	CYP2C8 *3	
Iran	Present study	0.18	0	
Portuguese*	[23]	0.012	0.198	<0.0001
African-American*	[11]	0.18	0.02	<0.0001
Ghana*	[24]	0.17	0	0.0004
Zanzibar*	[25]	0.14	0.02	0.0006
Burkina Faso	[22]	0.115	0.004	0.1601
Sweden*	[28]	NS	0.095	<0.0001
Spain*	[26]	NS	0.17	<0.0001
Czech Republic*	[27]	0.003	0.109	<0.0001

**: shows significant difference from our population*

5. CONCLUSION

We identified significant differences in *CYP2C8* allelic variation between Iranian populations and other ethnic groups. Future work will include identifying clinical challenges these variants may pose by evaluating the functional roles of *CYP2C8* polymorphisms. Specifically, the clinical and toxicological significance of altered *CYP2C8* expression and activity caused by genetic, epigenetic, and environmental factors requires further investigation.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

ACKNOWLEDGEMENTS

We thank the research deputy of Zanjan University of Medical Sciences for financial support of this project (PharmD thesis, grant number A-10-349-6).

COMPETING INTERESTS

The authors have declared that no competing interests exist.

REFERENCES

1. Nagata K, Yamazoe Y. Genetic polymorphism of human cytochrome p450 involved in drug metabolism. Drug Metab Pharmacokinet. 2002;17(3):167-89.
2. van der Weide J, Steijns LS. Cytochrome P450 enzyme system genetic polymorphisms and impact on clinical pharmacology. An Clin Biochem. 1999;36(6):722-9.

3. Brosen K, De Morais SM, Meyer UA, Goldstein JA. A multifamily study on the relationship between CYP2C19 genotype and s-mephenytoin oxidation phenotype. Pharmacogenetics. 1995;5(5):312-7.
4. Gardiner SJ, Begg EJ. Pharmacogenetics, drug-metabolizing enzymes and clinical practice. Pharmacol Rev. 2006;58(3):521-90.
5. Yasar U, Bennet AM, Eliasson E, Lundgren S, Wiman B, De Faire U, et al. Allelic variants of cytochromes P450 2C modify the risk for acute myocardial infarction. Pharmacogenetics. 2003;13(12):715-20.
6. Goldstein JA. Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. Br J Clin Pharmacol. 2001;52(4):349-55.
7. Garcia-Martin E, Martinez C, Ladero JM, Agundez JA. Interethnic and intraethnic variability of CYP2C8 and CYP2C9 polymorphisms in healthy individuals. Mol Diagn Ther. 2006;10(1):29-40.
8. Mancy A, Antignac M, Minoletti C, Dijols S, Mouries V, Duong NT, et al. Diclofenac and its derivatives as tools for studying human cytochromes P450 active sites particular efficiency and regioselectivity of P450 2Cs. Biochemistry. 1999;38(43):14264-70.
9. Suzuki A, Iida I, Tanaka F, Akimoto M, Fukushima K, Tani M, et al. Identification of human cytochrome P-450 isoforms involved in metabolism of R(+)- and S(-)-gallopamil utility of in *vitro* disappearance rate. Drug Metab Dispos. 1999;27(11):1254-9.
10. Daily EB, Aquilante CL. Cytochrome P450 2C8 pharmacogenetics a review of clinical studies. Pharmacogenomics. 2009;10(9):1489-510.
11. Dai D, Zeldin DC, Blaisdell JA, Chanas B, Coulter SJ, Ghanayem BI, et al. Polymorphisms in human CYP2C8 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid. Pharmacogenetics. 2001;11(7):597-607.
12. Muthiah YD, Lee WL, Teh LK, Ong CE, Ismail R. Genetic polymorphism of CYP2C8 in three Malaysian ethnics CYP2C8*2 and CYP2C8*3 are found in Malaysian Indians. J Clin Pharm Ther. 2005;30(5):487-90.
13. Niemi M, Backman JT, Neuvonen M, Neuvonen PJ. Effects of gemfibrozil, itraconazole, and their combination on the pharmacokinetics and pharmacodynamics of repaglinide potentially hazardous interaction between gemfibrozil and repaglinide. Diabetologia. 2003;46(3):347-51.
14. Tornio A, Niemi M, Neuvonen PJ, Backman JT. Trimethoprim and the CYP2C8*3 allele have opposite effects on the pharmacokinetics of pioglitazone. Drug Metab Dispos. 2008;36(1):73-80.
15. Wu X, Zuo J, Guo T, Yuan L. CYP2C8 polymorphism frequencies among Han, Uighur, Hui and Mongolian Chinese populations. Genet Test Mol Biomarkers. 2013;17(2):104-8.
16. Arnaldo P, Thompson RE, Lopes MQ, Suffys PN, Santos AR. Frequencies of Cytochrome P450 2B6 and 2C8 Allelic Variants in the Mozambican Population. Malays J Med Sci. 2013;20(4):13-23.
17. Rendic S, Di Carlo FJ. Human cytochrome P450 enzymes a status report summarizing their reactions, substrates, inducers and inhibitors. Drug Metab Rev. 1997;29(1-2):413-580.
18. Totah RA, Rettie AE. Cytochrome P450 2C8 substrates, inhibitors, pharmacogenetics and clinical relevance. Clin Pharmacol Ther. 2005;77(5):341-52.
19. Bahadur N, Leathart JB, Mutch E, Steimel-Crespi D, Dunn SA, Gilissen R, et al. CYP2C8 polymorphisms in Caucasians and their relationship with paclitaxel 6alpha-hydroxylase activity in human liver microsomes. Biochem Pharmacol. 2002;64(11):1579-89.

20. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16(3):1215.
21. Sawicki MP, Samara G, Hurwitz M, Passaro E. Human genome project. The American journal of surgery. 1993;165(2):258-64.
22. Parikh S, Ouedraogo JB, Goldstein JA, Rosenthal PJ, Kroetz DL. Amodiaquine metabolism is impaired by common polymorphisms in CYP2C8 implications for malaria treatment in Africa. Clin Pharmacol Ther. 2007;82(2):197-203.
23. Cavaco I, Piedade R, Gil JP, Ribeiro V. CYP2C8 polymorphism among the Portuguese. Clin Chem Lab Med. 2006;44(2):168-70.
24. Rower S, Bienzle U, Weise A, Lambertz U, Forst T, Otchwemah RN, et al. Short communication: high prevalence of the cytochrome P450 2C8*2 mutation in Northern Ghana. Trop Med Int Health. 2005;10(12):1271-3.
25. Kudzi W, Dodoo AN, Mills JJ. Characterisation of CYP2C8, CYP2C9 and CYP2C19 polymorphisms in a Ghanaian population. BMC Med Genet. 2009;10:124.
26. Martinez C, Garcia-Martin E, Blanco G, Gamito FJ, Ladero JM, Agundez JA. The effect of the cytochrome P450 CYP2C8 polymorphism on the disposition of (R)-ibuprofen enantiomer in healthy subjects. Br J Clin Pharmacol. 2005;59(1):62-9.
27. Pechandova K, Buzkova H, Matouskova O, Perlik F, Slanar O. Genetic polymorphisms of CYP2C8 in the Czech Republic. Genet Test Mol Biomarkers. 2012;16(7):812-6.
28. Yasar U, Lundgren S, Eliasson E, Bennet A, Wiman B, de Faire U, et al. Linkage between the CYP2C8 and CYP2C9 genetic polymorphisms. Biochem Biophys Res Commun. 2002;299(1):25-8.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=403&id=12&aid=3379