SCIENCEFORECAST
A digital science library

Research Article
Published: 05 Jun, 2020

SF Journal of Neurological Disorders and Rehabilitation

Comparing Genetic Polymorphisms of CYP2C9 and CYP2C19 in the Iranian Epileptic Patients and Healthy Subjects

*Nematollahi H[1], Salehi M[2], Saliminejad K[3] and Sadrai S[1]**

[1]Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

[2]Faculty of Medical Science, Isfahan University of Medical Sciences, Isfahan, Iran

[3]Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

Abstract

Purpose: Genetic variation in a gene coding for a drug-metabolizing enzyme can cause enzyme variants with high, low or no activity. For better and proper dose adjusting, it is valuable to know if distributions of polymorphism in epileptic and healthy subjects are the same or not.

Methods: 140 healthy and 100 patients with epilepsy were studied. The DNA was extracted from the peripheral blood leucocytes and genotyped for CYP2C9*1, CYP2C9*2, CYP2C9*3 as well as CYP2C19*1, CYP2C19*2, CYP2C19*3, CYP2C19*17 by PCR-RFLP assay.

Results: In patients and healthy subjects no CYP2C9*3 allele and in healthy subjects no CYP2C19*3/*3 genotype was detected. The frequency distribution of CYP2C9 and CYP2C19 gene polymorphism in patients was significantly different to healthy populations (p=0.008).

Conclusion: Human genetic polymorphisms in drug metabolizing enzymes in patient group have been shown to correlate with the risk of drug-related toxicity. The present study revealed polymorphism of CYP2C9 and CYP2C19 in epileptic patients and its significant difference of their distribution in comparison with healthy subjects.

Keywords: Pharmacogenetic; Epilepsy; Polymorphism; Phenytoin; Phenobarbital

OPEN ACCESS

*Correspondence:
Sima Sadrai, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
E-mail: sadrai@tums.ac.ir

Received Date: 09 May 2020
Accepted Date: 01 Jun 2020
Published Date: 05 Jun 2020

Citation: Nematollahi H, Salehi M, Saliminejad K, Sadrai S. Comparing Genetic Polymorphisms of CYP2C9 and CYP2C19 in the Iranian Epileptic Patients and Healthy Subjects. SF J Neurol Disord Rehabil. 2020; 1(1): 1003.

Abbreviations

Cyp: Cytochrome; PM: Poor Metabolizers; IM= Intermediate Metabolizers; EM=Extensive Metabolizers; UM= Ultra-rapid Metabolizers

Introduction

Pharmacogenetic is the study of the genetic basis for variation in drug response [1]. Genetic variations in drug target proteins, drug-metabolizing enzymes, and drug transporters can alter drug efficacy or drug side effects [2]. Cytochrome P450 comprise a superfamily of haemoproteins witch play an important role in the metabolism and elimination of a wide range of medications, as well as other xenobiotic [3-5]. The genes encoding CYP2C9 and CYP2C19 are polymorphically expressed, with 30 variant alleles for CYP2C9 and 21 for CYP2C19. Many of these variants, the most common being *2 and *3, are associated with decreased metabolism of the respective substrates. The frequency of polymorphic alleles shows marked inter-ethnic variation [6]. Genetic variation in a gene coding for a drug-metabolizing enzyme can cause enzyme variants with high, low or no activity. Thus, populations can be divided in to phenotypes of Poor Metabolizers(PM), Intermediate Metabolizers(IM), Extensive Metabolizers(EM) and Ultra-rapid Metabolizers (UM) [7].

Few studies have reported allelic frequency of CYP2C19*17 and its effect on clinical response to CYP2C19 substrates [8]. In many cases, CYP2C19*17 does increase enzyme activity, but when comparing the effect on multiple drugs, CYP2C19*17 is not always a clinically relevant contributor to drug metabolism [9].

CYP2C9 and CYP2C19 genes are located on chromosome 10q24 [10]. The nucleotide changes in the CYP2C9*2 and CYP2C9*3 alleles lead to changes in the amino acid sequence and in the CYP2C19*2 and CYP2C19*3 the nucleotide changes lead to a splicing defect and stop codon respectively, therefore to decreased enzyme activity [11].

Methods

100 patients with epilepsy and 140 healthy Iranian volunteers were studied. The research was approved by Tehran University of Medical Sciences Ethics Committee for the grants 89-03-33-11323 and 90-01-33-11366 and written informed consent was obtained from subjects. Demographic characteristics of the epileptic patients are demonstrated in Table1. For each subject, 5μl sample of venous blood was collected in an EDTA tube. The DNA was extracted from the peripheral blood leucocytes. PCR was performed in a 25μl reaction containing 10 pmol of forward and reverse primers, PCR buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$ and 1U of Taq DNA polymerase. The PCR conditions for CYP2C19 were as follows: an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 53°C for 40 seconds, extension at 72°C for 30 seconds. Final extension at 72°C for 5 min. also for CYP2C9 were follows: an initial denaturation for 2 min at 94°C followed by 35 cycles of 30s at 94°C, 10s at 60°C, 1 min at 72°C and a terminal extension for 7 min at 72°C. PCR products were digested with a specific restriction endonuclease. PCR products of CYP2C19*2, CYP2C19*3 were digested with SmaI and BamHI respectively, PCR products of CYP2C9*2, CYP2C9*3 were digested with Sau96I and StyI respectively. The digested PCR products were separated by 3% agarose gel electrophoresis. In the first phase of the study we did not study CYP2C19*17 but after reports of this gene we repeated the whole protocol considering CYP2C19*17 gene polymorphism in the samples.

Results

The CYP2C9/CYP2C19 genotype frequencies for both the patient and control groups are summarized in Table 3. We did not find any CYP2C9*3 allele.

The frequency distribution of CYP2C9 and CYP2C19 gene polymorphism in patients was significantly different to healthy populations (p=0.0082).

Discussion

There are major inter-individual differences in response to drugs and other xenobiotic. These are of genetic, epigenetic, environmental, and pathophysiological origin [12]. Human genetic polymorphisms in drug metabolizing enzymes have been shown to correlate with the risk of drug-related toxicity [13]. Genetic polymorphisms identified for members of CYP2C subfamily, i.e., CYP2C9, and CYP2C19 [14]. The CYP2C9*2 and CYP2C9*3 alleles have a lower frequency in the Asian and African populations [15].

Previous studies found that CYP2C9*2, CYP2C9*3, CYP2C19*2,

Table 1: Primers and endonucleases used for genotyping.

Allel	Primer set	Size (bp)	Detection
2C19*2	5′-AATTACAACCAGAGCTTGGC-3′(F) 5′-TATCACTTTCCATAAAAGCAAG-3′(R)	169	SmaI, 25ºC
2C19*3	5′-TATTATTATCTGTTAACTAATATGA-3′(F) 5′-ACTTCAGGGCTTGGTCAATA-3′(R)	329	BamHI, 37ºC
2C19*17 (-3402C>T)	5′-ATGACCTTGATCTGGCAATGG(F) 5′-TATTAGATACTGCCCAACTGTCTC(R)	516	MnlI, 37ºC
2C19*17 (-806C>T)	5′-CCCTTAGCACCAAATTCTCTGAG-3′(F) 5′-AGCAGCCTAAACATGAAATAGCT-3′(R)	569	LweI (SfaNI), 37ºC
2C9*2	5′-CACTGG CTGAAAGAGCTAACAGAG-3′(F) 5′-GTGATATGGAGTAGGGTCACCCAC-3′(R)	372	Sau96I, 37ºC
2C9*3	5′-AGGAAGAGATTGAACGTGTGA-3′(F) 5′-GGCAGGCTGGTGGGGAGAAGGCCAA-3′(R)	130	StyI, 37ºC

Table 2: Demographic profile of epilepsy patients.

Characteristic	Mean
Age (years)	29+/-15.2
Sex(male/female)	58/42
Height (cm)	149.5+/-23.7
Body weight (kg)	56.6+/-20.1
Dose (mg) Phenytoin Phenobarbital Compound	 100 100,15,10,60 100/50
Polytherapy	40
Monotherapy	60
Phenytoin	13
Phenobarbital	45
Compound	28
Manufacture 1 2 3 4 5	 33 2 12 9 44
Smoking	9%
Alcoholism	3%

Table 3: Genotypes of CYP2C19 and CYP2C9 in 100 patients and 140 healthy subjects before study of CYP2C19*17.

Genotype	n (%) (patients=100)	N (%) (healthy)
CYP2C19*1/*1	37	106 (75.7)
CYP2C19*1/*2	14	9 (6.4)
CYP2C19*2/*2	1	11 (7.8)
CYP2C19*1/*3	30	11 (7.8)
CYP2C19*2/*3	15	2 (1.4)
CYP2C19*3/*3	3	1 (0.7)
CYP2C9*1/*1	86	104 (74.3)
CYP2C9*1/*2	8	30 (21.4)
CYP2C9*2/*2	6	6 (4.3)
CYP2C9*1/*3	0	0
CYP2C9*2/*3	0	0
CYP2C9*3/*3	0	0

There is a significance difference between patients and healthy subjects with a p-level=0.0082.

Table 4: Genotypes of CYP2C19 in 100 patients and 140 healthy subjects after study of CYP2C19*17.

Genotype	n (%) (patients=100)	N (%) (healthy)
CYP2C19*1/*1	28	72(51.4)
CYP2C19*1/*2	10	17(12.1)
CYP2C19*2/*2	0	1(0.7)
CYP2C19*1/*3	18	11(7.8)
CYP2C19*2/*3	8	2(1.4)
CYP2C19*3/*3	1	0(0)
CYP2C19*1/*17	10	27(19.3)
CYP2C19*2/*17	10	2(1.4)
CYP2C19*3/*17	12	2(1.4)
CYP2C19*17/*17	3	6(4.3)

There is a significance difference between patients and healthy subjects with a p-level=0.0082.

Table 5: Genotype frequencies of CYP2C9 in different population.

Population	Genotype frequency (%)						Refs.
	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3	
Our study (patients)	86	8	0	6	0	0	-
Our study (healthy)	74.3	21.4	0	4.3	0	0	-
Iranian (south)	41.2	37.83	9.46	1.35	10.13	0	[20]
Mexican	94.9	2.02	3.03	0	0	0	[30]
Malay	86.5	0	11.94	0	0	1.49	[15]
Pakistan	85.8	0	11.7	0.8	0	1.7	[25]
Turkish	61.7	18.04	17.23	1	1.10	0.80	[26]
Romanian	63.96	21.3	14.8	0	0	0	[24]

Table 6: Genotype frequencies of CYP2C19 in different population.

Population	Genotype frequency (%)										Refs.
	*1/*1	*1/*2	*1/*3	*1/*17	*2/*2	*2/*3	*2/*17	*3/*3	*3/*17	*17/*17	
Our study (patient)	28	10	18	10	0	8	10	1	12	3	-
Our study (healthy)	51.4	12.1	7.8	19.3	0.7	1.4	1.4	0	1.4	4.3	-
Iranian	41.7	18.3	0	28.8	2.2	0	3.3	0	0	5.5	[8]
Iranian (random)	75	22	0	n	3	0	n	0	n	n	[7]
Iranian (Fars)	75	22	1.4	n	1.4	0		0	n	n	[21]
France	76 65	23	n	29	1	n	n	n	n	5%	[32]
Italian	79.4	18.8	1.6	n	0	0	n	0	n	n	[21]
Moroccan	9.1 3.03 10.87	84.85	75.76	69.57	6.06	n	n	21.21	n	19.56	[31]
Greek	76	22	0	n	2	0	n	0	n	n	[21]
Kosovar	45.72	18.37	n	26.06	2.13	n	3.41	n	n	4.27	[27]
Iranian (Mazandaran)	84	14	0	n	2	0	n	0	n	n	[22]
Chinese	36.7	38.2	5.8	n	5.8	11	n	1.4	n	n	[21]
Iranian (Turkaman)	37.9	42.1	9.3	n	9.3	0	n	1.4	n	n	[23]
Indian	35	55	0	n	10	0	n	0	n	n	[21]
Africans	66.1	29.8	0.7	n	2.7	0.4	n	0	n	n	[22]
South Indian	16.1	31.0	0	20.7	18.4	0	12.6	0	0	1.2	[29]

n: not studied

CYP2C19*3, largely decreased the catalytic efficiency of the enzyme.

In 2006 a novel variant allele, CYP2C19*17 was reported which is specified by a double mutation in promoter region (CYP2C19 -806C>T and -3402C>T), these mutations lead to increased gene transcription and therefore Ultra-Rapid Metabolizer phenotype (URM) [8,11].

Significance of CYP2C genotyping before drug treatment has been shown for patients treated with the antiepileptic drugs [16].

In our study the frequency distribution of CYP2C9 and CYP2C19 gene polymorphism in patients was significantly different to healthy populations (p=0.0082).

The CYP2C9/CYP2C19 polymorphism studied in other populations [17,18].

In a study of 200 healthy Iranian subjects CYP2C19*3 allele was not found [3].

Despite the availability of several antiepileptic drugs, in appropriate response to these drugs has become a concern in the treatment of this disease, [19,20] therefore the study of genes in response to these drugs appears to be required.

The CYP2C19*17 variant has been associated with ultra-rapid drug metabolism for two of its substrates, omeprazole and escitalopram, which might imply increase risk of therapeutic failure [28].

In Table 3 and 4 there we can see the frequencies of genotype frequencies without and with CYP2C19*17 and there is 35% of patients and 37% in healthy group with a CYP2C19 *17. It seems that for example a CYP2C19 3*17 activity is as a CYP2C19 1*1. The activity and effectiveness of different CYP2C19 *17 must be studied in future. In Tables 5 and 6 thee is a compare of our study with other similar studies.

Conclusion

The main difference is that the epileptic group have about 20% poor metabolizer in compare to healthy group with only about 10%. Due of this difference in the genotype frequencies of CYP2C19 in patients and healthy group, in dose adjustment this must be

considered.

Funding

This project was supported by two grants from Drug Design and Development Research Centre, Tehran University of Medical Sciences, Tehran, Iran (89-03-33-11323 and 90-01-33-11366). These grants are academic without financial benefit.

Authors' Contributions

Nematollahi H: Sample gathering, data analysis, manuscript preparation.

Salehi M: experimental guidance and oversight.

Saliminejad K: experimental guidance and oversight.

Sadrai S: Experimental conception, data interpretation, manuscript preparation.

Acknowledgement

Here with we thank Majid Ghasemi (Associate Professor of Neurology, Isfahan University of Medical Sciences) and Isfahan Epilepsy Association for announcing the patients to participate in this study.

References

1. Ellison N. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 2002.
2. Ma Q, Lu AY. Pharmacogenetics, pharmacogenomics, and individualized medicine. Pharmacol Rev. 2011; 63: 437-459.
3. Zand N, Tajik N, Hoormand M, SalekMoghaddam A, Milanian I. Allele frequency of CYP2C19 gene polymorphisms in a healthy Iranian population. IJPT. 2005; 4: 124-130.
4. Miners JO, Birkett DJ. Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. Br J Clin Pharmacol. 1998; 45: 525-538.
5. Ali ZK, Kim RJ, Ysla FM. CYP2C9 polymorphisms: considerations in NSAID therapy. Curr Opin Drug Discov Devel. 2009; 12: 108-114.
6. Rosemary J, Adithan C. The pharmacogenetics of CYP2C9 and CYP2C19: Ethnic variation and clinical significance. Curr Clin Pharmacol. 2007; 2: 93-109.
7. Zand N, Tajik N, Moghaddam AS, Milanian I. Genetic polymorphisms of cytochrome P450 enzymes 2C9 and 2C19 in a healthy Iranian population. Clin Exp Pharmacol Physiol. 2007; 34: 102-105.
8. Payan M, Tajik N, Rouini MR, Ghahremani MH. Genotype and allele frequency of CYP2C19*17 in a healthy Iranian population. Med J Islam Repub Iran. 2015; 29: 269-276.
9. Sanford JC, Guo Y, Sadee W, Wang D. Regulatory polymorphisms in CYP2C19 affecting hepatic expression. Drug Metabolism Drug Interact. 2013; 28: 23-30.
10. Kudzi W, Dodoo AN, Mills JJ. Characterisation of CYP2C8, CYP2C9 and CYP2C19 polymorphisms in a Ghanaian population. BMC Medical Genetics. 2009; 10: 124.
11. Borlak J, Thum T. Identification of major CYP2C9 and CYP2C19 polymorphisms by fluorescence resonance energy transfer analysis. Clin Chem. 2002; 48: 1592-1594.
12. Johansson I, Ingelman-Sundberg M. Genetic polymorphism and toxicology-with emphasis on cytochrome p450. Toxicological Sciences. 2010; 120: 1-3.
13. Mochizuki J, Murakami S, Sanjo A, Takagi I, Akizuki S, Ohnishi A. Genetic polymorphisms of cytochrome P450 in patients with hepatitis C virus-associated hepatocellular carcinoma. J Gastroenterol Hepatol. 2005; 20: 1191-1197.
14. 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: 25-28.
15. Jakovski K, Nestorovska AK, Labacevski N, Dimovski AJ. Characterization of the most common CYP2C9 and CYP2C19 allelic variants in the population from the Republic of Macedonia. Pharmazie. 2013; 68: 893-898.
16. Sugimoto K, Uno T, Yamazaki H, Tateishi T. Limited frequency of the CYP2C19* 17 allele and its minor role in a Japanese population. Brit J Clin Pharmacol. 2008; 65: 437-439.
17. Lee SY, Lee ST, Kim JW. Contributions of CYP2C9/CYP2C19 genotypes and drug interaction to the phenytoin treatment in the Korean epileptic patients in the clinical setting. J Biochem Mol Biol. 2007; 40: 448-452.
18. Rosemary J, Adithan C. The pharmacogenetics of CYP2C9 and CYP2C19: Ethnic variation and clinical significance. Curr Clin Pharmacol. 2007; 2: 93-109.
19. Sisodiya SM. Genetics of drug resistance in epilepsy.Curr Neurol Neurosci Rep. 2005; 5: 307-311.
20. Azarpira N, Namazi S, Hendijani F, Banan M, Darai M. Investigation of allele and genotype frequencies of CYP2C9, CYP2C19 and VKORC1 in Iran. Pharmacol Rep. 2010; 62: 740-746.
21. Tabari RG, Ataby OA, Marjani A. CYP2C19 genetic polymorphism of cytochrome p450 enzyme in Iranian fars ethnic group. Ann Res Rev Biol. 2014; 4: 1471-1479.
22. Shahabi-Majd N, Habashi B. Frequencies of two CYP2C19 defective alleles (CYP2C19* 2, and* 3) among Iranian population in Mazandaran Province. Res Mol Med. 2013; 1: 16-20.
23. Tabari RG, Marjani A, Ataby OA, Mansourian AR, Samai NM. Genetic polymorphism of cytochrome p450 (2C19) enzyme in Iranian Turkman Ethnic group. Oman Med J. 2013; 28: 237-244.
24. Sabin O, Trifa AP, Brusturean E, Buzoianu AD. Study of CYP2C9 and CYP2C19 polymorphisms in a Romanian epilepsy population. Human Veterinary Medicine. 2013; 5: 77-81.
25. Siddiqi AI, Khan DA, Khan FA, Naveed AK. Impact of CYP2C9 genetic polymorphism on warfarin dose requirements in Pakistani population. Pak J Pharm Sci. 2010; 23: 417-422.
26. Aynacioglu S, Brockmöller J, Bauer S, Sachse C, Güzelbey P, Öngen Z, et al. Frequency of cytochrome P450 CYP2C9 variants in a Turkish population and functional relevance for phenytoin. Brit J Clin Pharmacol. 1999; 48: 409-415.
27. Krasniqi V, Dimovski A, Bytyqi HQ, Eftimov A, Šimičević L, Božina N. Genetic polymorphisms of CYP2C9, CYP2C19, and CYP3A5 in Kosovar population. Arh Hig Rada Toksikol. 2017; 68: 180-184.
28. Wyllie E. Wyllie's Treatment of epilepsy Principles and practice. Wolters Kluwer Press. 2015.
29. Anichavezhi D, Rao UC, Shewade DG, Krishnamoorthy R, Adithan C. Distribution of CYP2C19* 17 allele and genotypes in an Indian population. J Clin Pharm Ther. 2012; 37: 313-318.
30. Dorado P, Sosa-Macias MG, Peñas-Lledó EM, Alanis-Bañuelos RE, Wong ML, Licinio J, et al. CYP2C9 allele frequency differences between populations of Mexican-Mestizo, Mexican-Tepehuano, and Spaniards. Pharmacogenomics. 2011; 11: 108-112.
31. Idrissi HH, Hmimech W, El Khorb N, Akoudad H, Habbal R, Nadifi S. A synergic effect between CYP2C19* 2, CYP2C19* 3 loss-of-function and CYP2C19* 17 gain-of-function alleles is associated with Clopidogrel

resistance among Moroccan Acute Coronary Syndromes patients. BMC Res Notes. 2018; 11: 46.

32. Cuisset T, Loosveld M, Morange PE, Quilici J, Moro PJ, Saut N, et al. CYP2C19*2 and*17 alleles have a significant impact on platelet response and bleeding risk in patients treated with prasugrel after acute coronary syndrome. JACC Cardiovasc Interv. 2012; 5: 1280-1287.