

Frequencies of two CYP2C19 Defective Alleles (CYP2C19*2, and *3) among Iranian Population in Mazandaran Province

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Abstract

Background: Cytochrome P450 2C19 (CYP2C19) is a polymorphically expressed enzyme that shows marked interindividual and interethnic variation. CYP2C19*2 and CYP2C19*3 are the most frequent identified defective alleles in Orientals and Caucasian poor metabolizers (PM). The aim of this study was to investigate the frequencies of CYP2C19*1, CYP2C19*2 and CYP2C19*3 alleles and CYP2C19 genotypes among Mazandarani ethnic group among Iranian Population.

Materials and Methods: The study was conducted on 103 unrelated healthy volunteers. DNA was extracted from leucocytes and analyzed by the PCR-RFLP protocol. The PCR product was digested with restriction enzymes (SmaI and BamHI) and then separated electrophoretically using polyacrylamide gel.

Results: Of the tested alleles, CYP2C19*1, and CYP2C19*2, but not CYP2C19*3, were detected. The frequencies for CYP2C19 alleles *1, *2, and *3 were 91%, 9.0%, and 0.0%, respectively. CYP2C19 genotypes *1/*1, *1/*2, *1/*3, *2/*2, *2/*3 and *3/*3 frequencies were 84%, 14%, 0.0%, 2.0%, and 0.0%, respectively.

Conclusion: The result of the present study shows that the two inactive alleles of CYP2C19 accounted for 9.0% of CYP2C19 alleles in our sample versus 8.8 - 40.1% reported in other populations. The studied alleles frequencies was significant difference between our sample and African and Eastern Asian populations.

Keywords: CYP2C19; Genotype; Alleles; Mazandaran; Iranian

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Introduction

Genetic polymorphisms of cytochrome P450 (CYP) enzymes in humans are the main cause of differences in the metabolic activities of therapeutic drugs (1-2). The major genetic polymorphisms affecting drug metabolizing enzymes activity of potential clinical relevance are those related to drug oxidation by CYP2D6, CYP2C9 and CYP2C19 (3-4). CYP2C19 exhibits high genetic polymorphism (www.cypalleles.ki.se/cyp2c19.htm), including two common defective variants. Single-base substitutions in the coding sequence of CYP2C19*2 and CYP2C19*3 lead to splicing defect and premature stop codon, respectively. Therefore these substitutions can cause null function of the enzyme. There are significant ethnic differences in

the frequency of these variants. CYP2C19*2 has been identified as the most common mutant allele of CYP2C19 among Caucasians and Filipinos, appearing in 9.1- 39% of the population, respectively (5-9). CYP2C19*3 is common in Oriental population but extremely rare in Caucasians and among African populations (9). The prevalence of PMs was reported to be 2-5% in Caucasians (8, 10, 11), 4-8% in Africans (11-12) and 11-23% in Orientals (11, 13, 15).

Although the allele and genotype frequencies of CYP2C19 have been studied extensively in most populations world-wide, limited information is available for those of the Iranian population (16-17). Here in, we examined the frequencies of

CYP2C19*1, CYP2C19*2, and CYP2C19*3 alleles and CYP2C19 genotypes among Mazandarani ethnic group of Iranian Population.

Materials and Methods

Subjects

One hundred and three unrelated healthy volunteers of Mazandarani origin residing in Mazandaran, province in the north of Iran, were enrolled in the study. The study protocol was approved by the Research Ethics Committee of Mazandaran University of Medical Sciences. Written consent forms were given from all the subjects.

Chemicals and drugs

Magnesium chloride, ethylenediaminetetraacetic acid, Tris-hydrochloric acid, and sodium chloride were purchased from Merck Chemical Company. Restriction enzymes and primers were prepared by Fermentas. Ultra-pure water was obtained using a Milli-Q water purification system.

Determination of CYP2C19 genotype -Genomic DNA extraction and PCR amplification

A 10 milliliters venous blood was obtained from each subject and transferred into sterile plastic tubes containing Na-EDTA and stored at -25 °C until to be processed. Lymphocytic genomic DNA was isolated according to previously published method (18). The DNA samples were stored at 4 °C. Allele-specific polymerase chain reaction was carried out to detect the CYP2C19*2, CYP2C19*3 alleles. The method of De Morais et al. (6, 19) was used to detect the CYP2C19*2 and for CYP2C19*3, respectively.

Briefly, a solution of 0.5 µl of 100 ng/µl genomic DNA was pipetted into 0.2 ml thin-walled PCR tubes. A master mix was prepared according to the number of samples to be genotyped. This comprised (per tube) 1µl of 20 µM of each of the primers in Table 1, 0.2 µl Taq® DNA polymerase, 0.7 µl of sterile water and 46.5µl of 'Reddy mix' (75 mM Tris-HCl pH 8.3, 1.5 mM MgCl₂, 0.2 mM each of dATP, dCTP, dGTP and dTTP).

DNA samples were initially denatured at 94 °C for 1 minute. DNA amplification was achieved by 35 cycles of denaturation (95 °C, 60 sec), annealing for 60 sec and extension (72 °C, 60 sec). Then, the samples were subjected to a final extension step in 72 °C for 5 min. Finally, a 169 bp, and 329 bp, PCR fragment were achieved for CYP2C19*2, CYP2C19*3 respectively. A 10 µl of aliquot of each PCR product was run on a 10% polyacrylamide gel to check for positive amplification.

Restriction of amplified samples

Restricted enzyme digestion was carried out as

recommended by the manufacture.

Table1. Sequences and Orientation of Primers Used in Polymerase Chain Reactions.

Alleles	Primers sequence	Orientation	References
CYP2C19*2	5'- AATTACAACCAGAGCTTGGC-3'	F	(19)
	5'- TATCACTTTCATAAAAGCA-3	R	
CYP2C19*3	5'- TATTATTATCTGTAACTAA-3'	F	(6)
	5'- ACTTCAGGGCTTGGTCAATA -3	R	

f = forward primer, r = reverse primer

10 µl of each CYP2C19*2, and CYP2C19*3 sample were digested using a mastermix of each enzyme (30 µl restriction enzyme + 200 µl buffer, aliquot 50 µl) according to the manufacturer. A 2.3µl aliquot of each SmaI and BamH1 master mix was added to 10µl of PCR product for each CYP2C19*2 and CYP2C19*3 samples, respectively. The reaction tubes were incubated for overnight at 37 °C prior to analysis on a 10% polyacrylamide gel. The gel was electrophoresed for 40 min at 200 volts in 1XTBE buffer and stained by 1µg/ml ethidium bromide.

Results

One hundred and three subjects were genotyped. Examples of digestion reactions for detection of CYP2C19*2, and CYP2C19*3 alleles are shown in Figure 1.

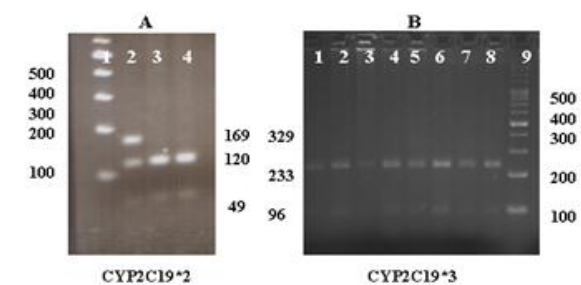


Figure 1. Gel pictures after polymerase chain reaction–restriction fragment length polymorphism. A) CYP2C19*2; Lanes 1,100bp DNA marker, lane 2 heterozygous for *2, lane 3 to 4, wild-type individuals. B) CYP2C19* 3; lanes 1 to 8, wild-type individuals, with 2 bands, 96 and 233 bp after 329 bp PCR product digestion with BamH1 restriction enzyme. Lane 9, 100bp DNA marker.

Of the alleles tested, CYP2C19*1, and CYP2C19*2, but not CYP2C19*3, were detected. The frequencies of the CYP2C19*1, and CYP2C19*2 alleles as well the genotype frequencies in the Mazandarani population are summarized in Table 2.

The frequency of polymorphic CYP2C19*2 allele was 9% in our population. Whereas 84% of patients were extensive metabolizer (EM) (all homozygous for the CYP2C19*1 allele, (CYP2C19*1/*1), 14% of subjects had a heterozygous intermediate metabolizer (IM) genotype (CYP2C19*1/*2). In 2% of subjects, a homozygous (CYP2C9*2/*2) PMs genotype was identified.

Table2. Allele and genotype frequencies of CYP2C19 in Mazandarani ethnic group among Iranian Population (n=103).

Variant allele	Allele frequency (%)	95% confidence interval
CYP2C19*1	91	87.4-93.3
CYP2C19*2	9	7.6-12.5
CYP2C19*3	0	-
Genotype	Frequency (%)	Anticipated phenotype (based on genotype)
CYP2C19*1/CYP2C19*	84	(Homozygous-EM)
CYP2C19*1/CYP2C19*2	14	(Heterozygous-IM)
CYP2C19*1/CYP2C19*3	0	(Heterozygous-IM)
CYP2C19*2/CYP2C1	2	(Homozygous -PM)
CYP2C19*3/CYP2C19*	0	(Homozygous -PM)

PM, poor metabolizer; EM,extensive metabolizer; IM, intermediate metabolizer.

Discussion

There are a number of ethnic groups living in various parts of Iran. These ethnic groups include Persian,

Azari, Turkmen, Kurd, Arab, Lor, Balouch, Gilaki and Mazandarani. Although CYP2C19 genetic polymorphisms have previously been assessed in a random Iranian population (16) and southern Iranians (17), there was no data in the Mazandarani ethnic group. The frequencies of the CYP2C19*2 alleles found in the Mazandarani ethnic group were very close to those found in Caucasian populations (9.1-15.9%) (5, 7, 20, 24) (Table3).

By contrast, the CYP2C19*2 alleles occur at a significantly higher frequencies in the Asian and East Asian countries (14-39%) (8, 9, 20, 25) (Table3). CYP2C19*2 allele frequency occur at relatively high frequencies in the African and African-Americans (13-25%) (7-9, 20, 26-29) (Table3). While CYP2C19*3 alleles is absent among different populations with Caucasian background, Europeans, Canadians-Australians, European (5, 8, 9,23,33,34), African and African-American populations (7, 9,26).

Table3. Allele and genotype frequencies of CYP2C19 in different population

Population	References	n	Allele frequency (%)			Genotype frequency (%)					
			*1	*2	*3	*1/*	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
Asians											
Present study	Present study	103	91	9	0	84	1	0	2	0	0
Iranian (random)	(30)	82	84.75	13.41	1.8	70.7	24.3	3.7	1.3	0	0
Iranian (southern)	(17)	147	86.73	13	1	74.14	24.4	0.68	0	0.68	0
Iranian (random)	(16)	200	86	14	0	75	22	0	3	0	0
Indian [31]	(25)	230	67	33	0	46	42	0	0.1	0	0
Gaza Strip	(32)	200	91.3	5.8	3	86.5	6	1.5	3	0.5	2
Tamilian	(20)	112	59.8	37.9	2.2	29.5	58	2.7	8	1.8	0
North Indians	(20)	121	70.3	29.7	0	47.9	44.6	0	7.4	0	0
Oriental*	(20)	368	61	30.5	8.5	38.4	36.7	8.4	8.7	6.5	1
Caucasians											
Russian	(24)	290	88.2	11.4	0.3	78.7	19	0.3	1.7	0.3	0
Italian	(5)	360	88.9	11.1	0	79.4	18.9	0	1.7	0	0
Greek	(5)	283	87	13	0	75.97	21.91	0	2.21	0	0
Slovenian	(22)	129	83.7	15.9	0.3	68.2	30	0.7	0	0	0
Croatian	(21)	200	85	15	0	73	24	0	3	0	0
Belgian	(7)	121	90.9	9.1	0	83.5	14.9	0	1.6	0	0
Caucasians**	(20)	837	86	14	0	74.8	23	0	2.2	0	0
Africans											
Beninese	(7)	111	87	13	0	73.9	26.1	0	0	0	0
Egyptian	(27)	247	88.8	11	0.002	78.56	20.24	0.40	0.80	0	0
Tanzanian	(26)	195	90	10	0	82	16.4	0	1.5	0	0
Africans	(20)	251	81.4	17.9	0.7	66.1	29.8	0	2.7	0.4	0

*(Japanese, Chinese, and Thai); ** (European Americans, Turks, Germans)

The frequency of CYP2C19*3 found in Mazandarani ethnic group in this study was (0%) similar to those showed in Caucasian, Africans and also previous reports from a random sample of Iranians (Table 3). By contrast, the CYP2C19*3 alleles occur at a relatively higher frequencies in the East Asian countries (8, 9, 20) (Table 3).

The frequency of poor metabolizers of CYP2C19 found in the present study (2%) was comparable to Caucasian populations (2-5%) (8, 10, 11). Dissimilarity CYP2C19 PM frequency is higher in Africans (4-8%) (11, 12) and in Orientals (11-23%) (11, 13-15). In conclusion, the tested allelic variants of CYP2C19 existed in the Mazandarani ethnic group

with frequencies are comparable to Caucasian populations with some differences among East Asians and Africans. It is hoped that our results will aid in understanding the ethnic diversity of the Iranian population, and offer a preliminary basis for more rational use of drugs that are substrates for CYP2C19 in this population.

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Conflict of interest: None declared.

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