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Original Research Article

Screening for CYP2C19 Gene variants in a healthy Jordanian population

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Abstract

Purpose: *To genotype healthy Jordanian population from three different provinces (Amman, Zarqa and Irbid) for cytochrome P4502C19 and to identify the allelic distribution of CYP2C19 variants in comparison with other findings around the world.*
Methods: *Healthy Jordanian volunteers were recruited from government hospitals. Two hundred and sixty volunteers were included in the study regardless of sex and age. CYP2C19*2, *3,*4, *5, and *6 alleles were studied using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique.*
Results: *The results show that the Jordanian population tested exhibited 9 genotypes out of the 21 expected CYP2C19 genotypes. CYP2C19*1/*1 and *2/*2 genotypes were the most prominent in the sample population, while CYP2C19*2/*5 was the least prevalent genotype. The frequencies of the CYP2C19 variants did not deviate from Hardy-Weinberg equilibrium. Allele frequency of CYP2C19*2 in the Jordanian population was statistically different from that found in most Europeans, North and South Americans, Africans, and some Asian ethnic communities but not with South-East Asian populations (China, Chinese-Taiwanese, and Philippines) and Australian Aborigines.*
Conclusion: *The findings of this study confirm the importance of CYP2C19 genotyping prior to drug therapy administration to achieve optimal dosage and cost-effective therapy.*

Keywords: *Cytochrome P450, RFLP-PCR, Allele frequency, Pharmacogenetics, Optimal dosage, Cost-effective therapy*

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INTRODUCTION

Individual variation in response to drugs is a substantial clinical problem that can be inherited. The genetically determined variability in drug response defines the research area known as pharmacogenetics [1].

One of the main directions in development of pharmacogenetics is identifying genes and allelic variants of genes that affect human's response to drugs [2]. Polymorphisms of genes encoding drug metabolism enzymes, drug transporters, and drug receptors, which are involved in drug responses, have been reported [3,4]. Genetic variation can account for as much as 95 % of variability in drug disposition and effect [5]. As

the main cause of the variation in drug response, attention has focused on genetic polymorphisms. Single nucleotide polymorphism (SNP) of DNA produces variation in drug response and has become a representative research target in pharmacogenetics [6].

It is known that many polymorphisms that influence drug response and which probably contribute significantly to phenotypic variation in drug response have significant allele frequency differences among racial or ethnic groups [3,7,8].

Of all genetic factor that affect drug treatment, those affecting the activity of the drug metabolizing enzyme cytochrome P450 are currently considered the quantitatively most important [6]. Humans have been estimated to have at least 57 different CYP genes, but the major drug metabolizing human P450 genes are CYP1A, CYP2B, CYP2C, CYP2D, CYP2E and CYP3A [9,10]. The CYP2C19 gene is located within a cluster of CYP genes on the 4th band of region 2 of the long arm of chromosome 10 (10q24) [11].

CYP2C19 exhibits high genetic polymorphism, including common defective variants. Single-base substitutions in the coding sequence lead to a splicing defect and premature stop codon, and therefore to null function of the enzyme. These variants together (CYP2C19*2 and CYP2C19*3) are responsible for the majority of the CYP2C19-related poor metabolism (PM) phenotypes in different populations [12].

Pharmacogenetics is a rapidly evolving field and a lot of information regarding genetic polymorphism is being generated for many ethnic groups, particularly Caucasians and Orientals. However, data on Arab populations is still sparse. There is a need to fill the gaps in pharmacogenetics knowledge pertaining to the Jordanian population. Thus, the aim of this study was to genotype healthy Jordanian people for cytochrome P4502C19, to identify the variant alleles of CYP2C19 and to compare the results with findings in other countries.

METHODS

Study group

Healthy Jordanian volunteers were randomly recruited from governmental hospitals of three provinces (Amman, Zarqa and Irbid). Two hundred and sixty volunteers were included in the study regardless of sex and age. All volunteers gave their written, informed consent. The study was approved by the Institutional Review Board (IRB) [AM/16/13/10/1200085] of the Hashemite University, Zarqa, Jordan which conforms to the World Medical Association Declaration of Helsinki [13].

CYP2C19 genotyping

Three milliliters of peripheral blood were withdrawn on ethylenediaminetetraacetic acid EDTA tube from each participant by venous puncture. DNA was extracted from 300 µL blood using a commercially available kit (Wizard Genomic DNA Purification Kit, Promega, Madison, USA). The CYP2C19*2, *3,*4, *5, and *6 alleles were studied using the PCR-RFLP technique. The PCR reactions were carried out in a BioRad thermocycler (Mycycler, BioRad, USA). All PCR primers are indicated from 5' to 3' end and are provided in Table 1. The primers sequences were based on published literature [14-17].

The amplification products were then digested overnight with the appropriate restriction endonuclease (Table 1). The digested PCR products were resolved by electrophoresis in 3 % agarose gels stained with ethidium bromide. The result of the electrophoresis was detected and documented using the CSL-MicroDoc and Canon digital camera (Cleaver-Scientific, UK).

Statistical analysis

The observed genotypes and allele frequencies were compared with those expected, in order to verify the Hardy-Weinberg equilibrium. Allelic frequencies and prevalence were compared between Jordanians and other populations using the test for differences between proportions. The statistical analysis has been performed using STATISITCA 7 analysis program (StatSoft Inc., Ok, USA). A value of $p < 0.05$ was considered statistically significant.

RESULTS

Population characteristics

The total number of subjects enrolled in the current study was 260. One hundred and fifty nine of them were females (61.2 %) and the other 101 (38.8 %) were males. The age of the subjects ranged from 18 to 59 years with a mean of 24.1 years. There was no significant difference (data not shown) between the mean age of females (24.3 years) and males (23.7 years) ($p > 0.05$).

Table 1: *CYP2C19* variants, PCR primers, PCR amplicons and their respective restriction digestion products

***CYP2C19* variant**	**Nucleotide sequence**	**PCR amplicon size (bp)**	**Restriction enzyme/ fragment size**
**2*	F: AATTACAACCAGAGCTTGGC R:TATCACTTTCCATAAAAGCAAG	168	SmaI/ 118 bp + 50 bp
**3*	F: AAATTGTTTCCAATCATTTAGCT R: ACTTCAGGGCTTGGTCAATA	271	BamHI/ 175 bp + 96 pb
**4*	F: ATTATATTAACAAGAGGAGAAGGCTGCA R: TTGGTTAAGGATTTGCTGACA	195	PstI/ 167 bp + 28 bp
**5*	F: TCCCTATGTTTGTTATTTCCAGG R: GAGCAGCCAGACCATCTGTG	229	BstX/ 203 bp + 26 bp
**6*	F: ATACAATGTAAATATGAATCTAAG R: CAGGACTCCAAATAAAAGATC	675	*Bsm*B I/ 490 bp + 185bp or *Pst*I/ 483 bp + 192 bp

Represents an allelic variant

CYP2C19 genotyping results

DNA extraction, PCR amplification and restricted PCR products were performed and recorded for all samples. Restriction products of the genomic DNA for the CYP2C19 variants (*2, *3, *4, *5 and *6) and their respective restriction enzymes were as shown in Table 1.

Genotype characteristics

The size of PCR amplicons and restriction digestion products were similar to what have been previously reported (Table 1 and Figure1). Genotypic and allelic frequencies for the six CYP2C19 variants were determined according to the count method (Table 2).

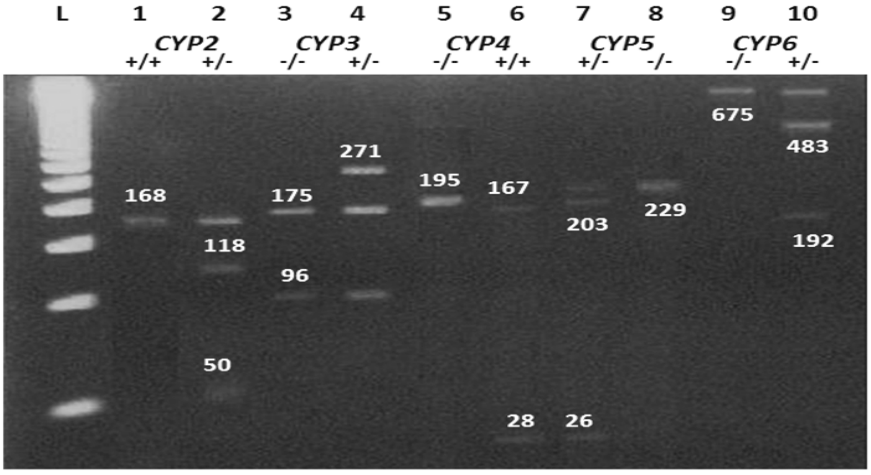

Figure 1: Representative gel electrophoresis showing the analysis of *CYP2C19* variants. Lane L: 50bp DNA molecule marker, lane 1: *CYP2C19*2* homozygote; lane 2: *CYP2C19*2* heterozygote; lane 3: *CYP2C19*3* wild type[a]; lane 4: *CYP2C19*3* heterozygote; lane 5: *CYP2C19*4* wild type, lane 6: *CYP2C19*4* homozygote; lane 7: *CYP2C19*5* heterozygote, lane 8: *CYP2C19*5* wild type; lane 9: *CYP2C19*6* wild type; lane 10: *CYP2C19*6* heterozygote. [a]: Wild type: *CYP2C191*/*1*;*represents an allelic variant

Table 2: *CYP2C19* genotypic and allelic frequencies in the Jordanian population

***CYP2C19* Genotype Frequency**	**1/*1*	**1/*2*	**1/*6*	**2/*2*	**2/*3*	**2/*4*	**2/*5*	**2/*6*	**4/*4*
	0.5731	0.0077	0.0269	0.3038	0.0154	0.0154	0.0038	0.0231	0.0308
***CYP2C19* Allele Frequency**	**1*	**2*	**3*	**4*	**5*	**6*			
	0.5904	0.3365	0.0077	0.0385	0.0019	0.0250			

Represents an allelic variant

This study has demonstrated a wide presence of *CYP2C19*1* allele in 158 unrelated healthy Jordanian individuals of which 149 (94.30 %) were *CYP2C19*1/*1* homozygotes, 2 (1.27 %) were *CYP2C19*1/*2* heterozygotes and 7 (4.43 %) were *CYP2C19*1/*6* heterozygotes. On the other hand, *CYP2C19*2* allele was reported in 96 healthy unrelated Jordanian individuals, of which 79 (82.29 %) were *CYP2C19*2/*2* homozygotes, 2 (2.08 %) were *CYP2C19*1/*2* heterozygotes, 4 (4.17 %) were *CYP2C19*2/*3* compound heterozygotes, 4 (4.17 %) were *CYP2C19*2/*4* compound heterozygotes, 1 (1.04 %) was *CYP2C19*2/*5* compound heterozygote, and 6 (6.25 %) were *CYP2C19*2/*6* compound heterozygotes, Furthermore, the study has shown the presence of *CYP2C19*4/*4* homozygosity in 8 (3.08 %) individuals. Therefore, 104 (40%) individuals – 96 *CYP2C19*2* allele homozygotes and heterozygotes (92.31 %) and 8 (7.69 %) *CYP2C19*4* allele homozygotes – are predicted to be poor metabolizers.

In addition, Table 2 shows that the Jordanian population tested exhibited nine genotypes out of the 21 expected *CYP2C19* genotypes. *CYP2C19*1/*1* and **2/*2* genotypes were the most prominent genotypes in the Jordanian sample population with a frequency of 0.5731 and 0.3038, respectively. *CYP2C19*2/*5* was the least prevalent genotype with a frequency of 0.0038. Furthermore, Table 2 shows the allelic frequencies of the six *CYP2C19* variants. *CYP2C19*1* and **2* were the most dominant variants with allelic frequencies of 0.5904 and 0.3365, respectively. *CYP2C19*5* was the least dominant variant with an allelic frequency of 0.0019. The frequencies of the *CYP2C19* variants in the Jordanian population were found not to deviate from the Hardy-Weinberg equilibrium.

Comparison between Jordanian allelic frequencies of *CYP2C19*2* and **3* alleles and that of other ethnic populations worldwide is shown in Table 3 [18,19]. Allele frequency of *CYP2C19*2* in the Jordanian population was statistically different ($p < 0.05$) from that found in most of the European, North and South Americans, Africans, and some Asian ethnic communities. No significant differences regarding the *CYP2C19*2* allele frequency were found when comparing Jordanians to South-East Asian populations (China, Chinese-Taiwanese, and Philippines) and Australian Aborigines. In the case of *CYP2C19*3* allele, a lower distribution as in Jordanians have been found in European, North and South America, Africa, and some South-East Asian ethnic groups. Compared to the Jordanian population, a significantly higher allelic frequency of *CYP2C19*3* allele was found in East-South Asian ethnic groups (China, Chinese-Taiwanese, Philippines, Japan and Korea), Australian Aborigines and Oceanians ($p < 0.05$). In this study, no statistical analysis was carried out for *CYP2C19*4, *5, and *6* variants due to the absence of data for most of the populations tested [18,19].

DISCUSSION

Various studies indicated the genetic basis for interethnic and inter-individual variability in the metabolism of CYP2C19 substrates [20,21]. This leads to dividing individuals according to their CYP2C19 genotype and the associated CYP2C19 enzyme activity into four categories: Extensive, Intermediate, Poor and Ultrarapid metabolizers, according to individuals' response to drug therapy [6].

The distribution of CYP2C19*2 allele in the Jordanian population is consistent with the relatively high frequency of CYP2C19*2 allele worldwide [22], suggesting that CYP2C19*2 was present in humans way before the separation of humans into different distinctive populations [23]. However, the higher frequency of such variant in the Jordanian population compared to some regional countries such as Saudi Arabia, Egypt, Gaza Strip (Palestine), and Turkey [19] may be hypothesized to be the result of emigration and admixture between the native Jordanians and different populations such as the Circassians, Palestinians, Syrian, Iraqi, Lebanese, Egyptians and other ethnic Arab populations. On the other hand, the distribution of CYP2C19*3 allele in the Jordanian population was found to be similar to its distribution within the international populations except the south-East Asians, Australian Aborigines and Oceanians [19], suggesting that this allele may be specific to these populations and that it occurred quite recently, after the separation of Caucasian and Oriental groups [23,24].

Limitations of the study

One major limitation of the current study is the scarce of information regarding CYP2C19 allele frequencies in Arab populations in order to make any kind of comparison. In addition, allele frequencies of CYP2C19*4, *5 and *6 in other populations are also lacking which made it impossible to draw any conclusion regarding these genotypes.

Table 3: Comparative *CYP2C19*2* and **3* allele frequencies in various population

Population	Sample size	*CYP2C19*2*f Frequency	*P*-value	*CYP2C19*3*f Frequency	*P*-value
Jordan	260	0.3365	-	0.0077	-
Europe					
Faroe Islands	310	0.188	0.00001	0.0000	0.1406
Denmark	239	0.16	0.00001	0.0000	0.1747
Germany	328	0.159	0.00001	0.0020	0.1947
Croatia	200	0.15	0.00001	0.0000	0.1588
Sweden	83	0.14	0.00001	0.0010	0.4183
Russia	290	0.114	0.00001	0.0030	0.3011
Italy	360	0.111	0.00001	0.0000	0.0577
French Caucasians	172	0.0013	0.00001	0.00003	0.1908
Romania	200	0.137	0.00001	0.0000	0.1588
Belgium	121	0.091	0.00001	0.0000	0.2704
Portugal	153	0.13	0.00001	0.0000	0.2154
North America					
European American	87	0.13	0.00001	0.0000	0.3498
African American	75	0.25	0.0033	0.0000	0.3858
Canadian Inuit	89	0.11	0.00001	0.0000	0.3443
Canadian Native Indian	115	0.191	0.00001	0.0000	0.2826
South America					
Colombia	189	0.087	0.00001	0.0000	0.1687
Bolivia	778	0.078	0.00001	0.0010	0.0276
Africa					
Tanzania	251	0.18	0.00001	0.0100	>0.999
Ethiopia	114	0.14	0.00001	0.0300	0.1580
Benin	111	0.13	0.00001	0.0000	0.2911
Egypt	247	0.109	0.00001	0.0020	0.2482
Zimbabwean	87	0.13	0.00001	0.0000	0.4123
Venda	78	0.22	0.0005	0.0000	0.3759
Asia					
Saudi Arabia	97	0.15	0.00001	0.0000	0.3236
Palestine (Gaza Strip)	200	0.0577	0.00001	0.0288	0.1352
Jewish Israel	140	0.15	0.00001	0.0100	0.2359
Iran	200	0.14	0.00001	0.0000	0.1568
Turkey	404	0.12	0.00001	0.0040	0.3424
China	121	0.455	0.784	0.0450	0.0276
Chinese-Taiwanese	63	0.32	0.084	0.0500	0.032
Philippines	52	0.39	0.5069	0.0800	0.0017
North India	121	0.297	0.0092	0.0000	0.2704
Japan	217	0.274	0.0002	0.1080	0.00001
Korea	103	0.209	0.00001	0.1170	0.00001
Australia and Oceania					
Australian Aborigines	227	0.355	0.0568	0.1430	0.00001
Vanuatu + other pacific ocean islands	5538	0.633	0.00001	0.1440	0.00001

*******Represents an allelic variant*

CONCLUSION

Earlier studies indicate that CYP2C19 polymorphic nature plays a crucial role in the pharmacokinetics of drug therapy, thus suggesting the importance of CYP2C19 genotyping prior to drug administration to achieve optimal dosage adjustment and cost-effective therapy. The findings of this study confirm the importance of CYP2C19 genotyping prior to drug therapy administration to achieve optimal dosage and cost-effective therapy.

DECLARATIONS

Acknowledgement

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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