RESEARCH NOTE

Characterization of drug-metabolizing enzymes CYP2C9, CYP2C19 polymorphisms in Tunisian, Kuwaiti and Bahraini populations

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Introduction

Knowledge of allelic frequency distribution in cytochrome P450 genes within populations can be useful in explaining therapeutic failure. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was applied for the detection of different allelic variants located in *CYP2C19* and *CYP2C9* genes in Tunisian, Kuwaiti and Bahraini populations. The frequencies of the CYP2C9*2, CYP2C9*3, CYP2C19*2 and CYP2C19*17 alleles in 258 Tunisian subjects were 0.139, 0.081, 0.090 and 0.213, respectively. The frequencies of CYP2C19*2 and CYP2C19*17 alleles were 0.105 and 0.250 in 100 Kuwaiti and 0.146 and 0.273 in 75 Bahraini subjects, respectively. We concluded that Tunisians are most similar to Europeans and Middle Easterners with regard to the allelic frequencies of CYP2C9 and CYP2C19 variants. Linkage disequilibrium (LD) analyses demonstrate that CYP2C19*17 is a determinant allele for a haplotype exhibiting an efficient CYP2C substrate metabolism in Tunisia. We believe that this is the first work that reports these variants among those populations.

The cytochrome P450 enzymes (CYPs) are a subfamily of hemoproteins, playing a critical role in the metabolism of many drugs. Several genetic polymorphisms which depend on ethnic groups can alter CYP activity and then affect the drug efficacy. Thus, subjects can be classified as poor metabolizers (PM), extensive metabolizers (EM) and ultrarapid metabolizers (UM) (Kurdzi *et al.* 2009).

The CYP2C9 enzyme which constitutes 50% of the CYP2C subfamily, catalyses the oxidation of a large number of non-steroidal anti-inflammatory drugs, antidepressant, warfarin and phenytoin (Allabi *et al.* 2003). Two polymorphisms, 430C>T and 1075A>C resulting in the CYP2C9*2 and CYP2C9*3 alleles, respectively, are associated with decreased metabolism of CYP2C9 substrate. Therefore, the frequency of the CYP2C9*2 allele leading to the substitution of arginine by cysteine (at position 144), ranges between 8 and 12% among Europeans, while it is lower in black Africans and absent in east Asians (Scordo *et al.* 2004). The variant allele CYP2C9*3 frequency, causing the substitution of isoleucine by leucine (at position 359), ranges between 3 and 8% among Europeans (Yousef *et al.* 2012).

Although, the CYP2C19 enzyme constitutes 16% of the CYP2C subfamily, it is a clinically important enzyme that metabolizes a wide range of drugs such as clopidogrel, omeprazole, diazepam and proguanil. The CYP2C19*2, a 681G>A nucleotide substitution in exon 5 resulting in an aberrant splice site, accounts for 75% of the defective alleles in Asians and 93% in Caucasians (Kurose *et al.* 2012). The CYP2C19*3 variant allele, which produces a premature codon stop at position 636 of exon 4, is rare among Caucasians and mainly found in Asian subjects (Allabi *et al.* 2003). Whereas, the CYP2C19*17, a −806C>T nucleotide substitution in the 5′ flanking region is associated with increased enzymatic function resulting in ultrarapid metabolism of CYP2C19 substrates (Sibbing *et al.* 2010). The rapid phenotype has been explained by a recruitment of transcription factor(s) in the gene promoter region causing an increased transcriptional rate.

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In this study, for the first time we report the allele frequencies of the main allelic variants CYP2C9*2, CYP2C9*3, CYP2C19*2 and CYP2C19*17 and investigate the LD between those polymorphisms in Tunisian population. Further, we report the prevalence of CYP2C19*2 and CYP2C19*17 polymorphisms in Kuwaiti and Bahraini populations.

Material and methods

Study subjects

A sample of 258 unrelated healthy subjects from southern Tunisia was recruited. The participants were students and staff at the Centre of Biotechnology of Sfax and the Cardiology Service of the Sfax Hedi Chaker University Hospital (mean age of 51.83 ± 17.17 years). In addition, 100 Kuwaiti (mean age 39.1 ± 17.1 years) and 75 Bahraini (mean age 36.25 ± 9.78 years) were collected and genotyped. An informed consent was obtained from all subjects.

Genotyping

Genomic DNA was extracted from EDTA anticoagulated peripheral blood samples using the standard phenol–chloroform method (Marcadet *et al.* 1987).

For the determination of CYP2C19*2 and CYP2C19*3 variant alleles, the protocol of PCR-RFLP described by Azarpira *et al.* (2010) was used. To identify the CYP2C19*17 variant allele, a nested PCR approach was used followed by restriction enzyme analysis with *Nsi*I as previously described by Vicente *et al.* (2014).

Genotyping for the CYP2C9*2 and CYP2C9*3 allelic variants of *CYP2C9* gene was performed by the conditions previously described by Azarpira *et al.* (2010).

Statistical analysis

Fisher's exact or the χ^2 test was used to test the deviation from the Hardy–Weinberg equilibrium (HWE) and to assess differences in allele frequencies between Tunisian and other populations including Kuwaiti and Bahraini ones. The level of statistical significance was set at $P < 0.05$.

Haplotype construction and LD analysis

The software Haploview was used to visualize the structure of pairwise LD between the single-nucleotide polymorphism (SNPs) in terms of Lewontin's D′. The squared correlation coefficient between allele frequencies (r^2) expressed as a function of D′. Haplotypes were inferred using the software package PHASE ver. 2.1. All the SNPs with minor allele frequencies <0.01% were excluded and the minimum haplotype frequency was set at 1%. Haplotype blocks were defined using the 'four gamete rules' incorporated in the Haploview program. The confidence interval (CI) minima for strong LD were set between 0.7 and 0.98.

Results and discussion

In order to ensure accuracy, we regenotyped 60 samples by the Sequenom MassARRAY platform, located at the Spanish National Genotyping Center at Santiago de Compostela, following the manufacturer's instructions. This genotyping platform has an average accuracy of 99.9% and is subjected to regular quality control procedure. We have obtained the same genotyping result for each selected sample by both the methods (PCR-RFLP and Sequenom MassARRAY).

CYP2C19

All genotype distributions were in HWE in the southern Tunisian, Kuwaiti and Bahraini samples (table 1). A higher frequency of EM was observed in Tunisian (0.837) population than in Kuwaiti (0.5) and Bahraini (0.425) populations. Whereas, a lower frequencies of IM and UM were observed in Tunisian population (0.128 and 0.031) than in Kuwaiti (0.350 and 0.120) and Bahraini (0.333 and 0.160) populations (table 1).

The frequency of the CYP2C19*2 allele in the Tunisian population was 0.090, which is in a range comparable with those in other African populations. In addition, no statistically significant difference was found between Tunisian and European as well as Middle Eastern including Kuwaiti and Bahraini populations ($P > 0.05$) (table 2). Whereas the highest frequencies of this variant were recorded for Afro-American (0.250) and Asian populations (table 2).

On the other hand, the CYP2C19*3 allele was not detected in Tunisian population, which is consistent with findings within Europeans and European–Americans. This variant was also absent in African, Afro–American and the Middle Eastern populations. In contrast, other studies suggested that CYP2C19*3 was a relatively rare allele in Ethiopian, Egyptian, Turkish, Lebanese, German, Dutch, Spanish and Slovenian populations (table 2). However, the CYP2C19*3 allele has been regarded as Asian mutation and accounted for the remaining alleles in Asian PMs after genotyping for CYP2C19*2 (ranging from 0.04 in Thailand to 0.124 in Japan) (table 2). This defect was noticed in different ethnic groups at a similar and relatively high frequency, implying that those mutations were relatively old and occurred before the Black, Asian and Caucasian racial groups split (Scordo *et al.* 2004).

The CYP2C19*17 allele was found with a frequency of 0.213 in the Tunisian population, similar to that found in the European subjects. However, this allele was rarely found in eastasian populations. In addition, the frequency of the CYP2C19*17 allele reported in Kuwaiti (0.250) and Bahraini (0.273) populations was similar to that found in Tunisian, and other European populations (table 2).

For a good clinical practice, it might be relevant to analyse three CYP2C19 alleles (*2, *3 and *17) since CYP2C19*2 and CYP2C19*3 are frequently found in Asian populations,

Table 1. Allele and genotype frequencies of CYP2C19 in different studied populations.

Allele frequency dbSNP ID	SNP (variant allele)	Tunisian ($n = 258$)			Kuwaiti ($n = 100$)			Bahraini ($n = 75$)		
		N (Freq)	95% CI	HWE *P* value	*N* (Freq)	95% CI	HWE *P* value	*N* (Freq)	95% CI	HWE *P* value
rs12248560 (−806C>T)	CYP2C19*17 (T)	110 (0.213)	0.176–0.249	0.083	50 (0.250)	0.194–0.314	0.018 (0.893)	41 (0.273)	0.208–0.349	0.123 (0.725)
rs4244285 (681G>A)	CYP2C19*2 (A)	46 (0.090)	0.064–0.115	0.210	21 (0.105)	0.069–0.155	4.07 (0.043)	22 (0.146)	0.098–0.212	0.127 (0.721)
rs4986893 (636G>A	CYP2C19*3 (A)	0	0	0.5	NA	NA	NA	NA	NA	NA
Genotype frequency										
Phenotype	Genotype	*N*	Freq	95% CI	*N*	Freq	95% CI	*N*	Freq	95% CI
	CYP2C19*1/*1	122	0.473	41–53.5	41	0.410	0.318–0.508	25	0.333	0.236–0.446
EM	CYP2C19*1/*17	83	0.322	26.3–38	6	0.060	0.025–0.127	5	0.066	0.025–0.150
	CYP2C19*2/*17	11	0.043	1.7–6.8	3	0.030	0.006–0.088	2	0.026	0.001–0.097
IM	CYP2C19*1/*2	33	0.128	6.8–16.9	35	0.350	0.263–0.447	25	0.333	0.236–0.446
UM	CYP2C19*17/*17	08	0.031	0.9–5.2	12	0.120	0.068–0.199	12	0.160	0.092–0.260
PM	CYP2C19*2/*2	01	0.003	0.3–0.9	3	0.03	0.006–0.088	6	0.080	0.034–0.166

CI, confidence interval; dbSNP ID, database SNP identifier; EM, extensive metabolizers; Freq, frequency; HWE, Hardy–Weinberg equation; IM, intermediate metabolizers; N, number; NA, not available; PM, poor metabolizers; UM, ultrarapid metabolizers.

whereas the CYP2C19*17 allele is more frequently found in European and African populations (Kim *et al.* 2010).

CYP2C9

All genotype distributions were in accordance with the HWE (table 3). The CYP2C9*2 allele frequency in the Tunisian population was 0.139, which is very close to those reported in European (from 0.099 in Norway to 0.165 in Croatia) and Middle Eastern populations (from 0.074 in Oman to 0.133 in Lebanon), but higher than those found in other studies regarding Asians, Afro–Americans and Africans (table 2). However, this variant was not detected in West African (Ghana and Benin) and East Asian populations (table 2). Regarding the CYP2C9*3 variant, Tunisian subjects showed a frequency of 0.081 similar to those found in European population but higher than those found in Asian, Afro-American and African populations. In addition, this allele frequency was similar to that reported in Middle Eastern population except for Saudi Arabia (0.023) and Oman (0.029), but the variant was absent in north of Iran (table 2).

Genetic polymorphisms of CYP2C9 and CYP2C19 genes have been shown to have clinical consequences resulting in the toxicity effects of some drugs on the affected individual, and may alter the efficacy of other drugs. For example, the CYP2C19*2 polymorphism has been associated with higher levels of ADP-induced platelet aggregation values in clopidogrel-treated patients and consequently, a higher risk of adverse cardiovascular events (Sibbing *et al.* 2010). Besides, a severe case of phenytoin intoxication has been reported by Ninomiya *et al.* (2000), in a heterozygous patient for CYP2C9*3 (*1/*3) with epilepsy who complained of diplopia and ataxia while receiving a low maintenance dose (187.5 mg/d; phenytoin serum concentration, 32.6 mg/L).

LD and haplotype inference

The calculations show complete LD ($D' = 1$) among CYP2C19*17 and CYP2C19*2 alleles ($r^2 = 0.025$; LOD: log of Odds = 1.51). The same result was found in other populations: south Indian, Chinese, European and African (Gurbel *et al.* 2010). Complete LD was also observed among CYP2C19*2 and two CYP2C9 alleles (CYP2C9*2 and CYP2C9*3) with a correlation coefficient (r^2) calculated as 0.015 (LOD = 1.42). The analysis also exhibits a strong LD between CYP2C9*2 and CYP2C9*3 alleles ($r^2 = 0.014$; LOD = 0.61). One block was identified that covers 199 kb and was assessed by CYP2C19*2, CYP2C9*2 and CYP2C9*3 polymorphisms.

Only seven of the 16 identified haplotypes had frequencies greater than 1% and together accounted for ∼98% of the overall CYP2C cluster haplotypic diversity (table 4). The most frequent haplotype (no. 1) with an inferred frequency of 53% carried the CYP2C19*1 and CYP2C9*1 wild-type alleles. The second most frequent haplotype (no. 2) with an inferred frequency of 17% was composed of CYP2C19*17 and CYP2C9*1 alleles, and represented 83.8% (88 out of 105) of all predicted CYP2C19*17-containing haplotypes. These haplotypes exclusively carry none or just a single SNP (mutant allele) except for haplotype no. 6 (2%) and no. 7 (1%), which are characterized by two SNPs. These are in accordance with the notion that SNPs are introduced to the genome as a single mutation occurring at a single point in time in a single individual and vertically propagated to the population (Pedersen *et al.* 2010).

We found that CYP2C19*17 is predicted to be almost exclusively present together with wild-type alleles of CYP2C9, thus mediating rapid metabolism of CYP2C19 substrates and normal metabolism of CYP2C9 substrates. This result is in agreement with that reported in European

Table 2. Comparison of allele frequencies of CYP2C9 and CYP2C19 polymorphism between Tunisian and different ethnic populations.

Country	CYP2C19 allele frequency				CYP2C9 allele frequency			References
	N	*2	*3	*17	*N*	*2	*3	
Africa								
Northern								
Tunisia	258	**0.090**	**0**	**0.213**	258	**0.139**	**0.081**	**Current study**
Eastern								
Ethiopia	228	0.136[b]	0.018[a]	NA	150	0.040[a]	0.020[a]	Persson *et al.* (1996)
Western								
Benin	111	0.130[b]	0[b]	NA	111	0[a]	0[a]	Allabi *et al.* (2003)
Ghana	204	0.060[b]	0[b]	NA	204	0[a]	0[a]	Kurdzi *et al.* (2009)
Southern								
South Africa	985	0.160[b]	0[b]	NA	923	0[b]	NA	Dandara *et al.* (2011)
Middle Eastern								
Egypt	247	0.110[b]	0.002[b]	NA	247	0.120[b]	0.062[b]	Hamdy *et al.* (2002)
Iran (north)	200	0.140[b]	0[b]	NA	200	0.127[b]	0[a]	Azarpira *et al.* (2010)
Jordan	158	0.123[b]	0[b]	NA	263	0.135[b]	0.068[b]	Yousef *et al.* (2012)
Lebanon	161	0.130[b]	0.031[a]	NA	161	0.122[b]	0.096[b]	Kurose *et al.* (2012)
Saudi Arabia	194	0.150[b]	0[b]	NA	131	0.133[b]	0.023[a]	Goldstein *et al.* (1997) Alzahrani *et al.* (2013)
Turkey	404	0.121[b]	0.004[b]	NA	499	0.106[b]	0.100[b]	Kurose *et al.* (2012)
Kuwait	**100**	**0.105**[b]	**NA**	**0.250**[b]		NA	NA	**Current study**
Bahrain	**75**	**0.146**[b]	**NA**	**0.273**[b]		NA	NA	**Current study**
Europe								
Northern								
Denmark	276	0.150[b]	NA	0.201[b]	276	0.121[b]	0.053[b]	Kurose *et al.* (2012)
Norway	309	0.152[b]	NA	0.220[b]	309	0.099[b]	0.065[b]	Kurose *et al.* (2012)
Sweden	185	0.160[b]	0[b]	0.200[b]	1894	0.108[b]	0.070[b]	Kurose *et al.* (2012)
UK	230	0.152[b]	NA	NA	230	0.157[b]	0.072[b]	Kurose *et al.* (2012)
Western								
Belgium	121	0.091[b]	0[b]	NA	121	0.100[b]	0.074[b]	Kurose *et al.* (2012)
Germany	423	0.154[b]	0.002[b]	0.256[b]	118	0.140[b]	0.051[b]	Kurose *et al.* (2012)
Netherlands	765	0.133[b]	0.002[b]	NA	284	0.127[b]	0.069[b]	Kurose *et al.* (2012)
Southern								
Croatia	200	0.150[b]	0[b]	NA	200	0.165[b]	0.095[b]	Kurose *et al.* (2012)
France	28	0.143[b]	0[b]	NA	151	0.150[b]	0.080[b]	Yang *et al.* (2003)
Greece	283	0.130[b]	0[b]	0.196[b]	283	0.129[b]	0.081[b]	Kurose *et al.* (2012)
Italy	360	0.111[b]	0[b]	NA	882	0.135[b]	0.090[b]	Kurose *et al.* (2012)
Portugal	126	0.140[b]	NA	NA	254	0.132[b]	0.080[b]	Kurose *et al.* (2012)
Slovenia	129	0.159[b]	0.004[b]	NA	129	0.120[b]	0.062[b]	Kurose *et al.* (2012)
Spain	282	0.128[b]	0.003[b]	0.149[b]	282	0.133[b]	0.077[b]	Vicente *et al.* (2014)
Eastern								
Russia (in European part)	352	0.131[b]	NA	NA	729	0.115[b]	0.061[b]	Kurose *et al.* (2012)
Asia								
Eastern								
China	1008	0.292[a]	0.042[a]	0.010[a]	1979	0.001[a]	0.037[a]	Kurose *et al.* (2012)
Japan	1944	0.293[a]	0.124[a]	0.011[a]	2559	0[a]	0.029[a]	Kurose *et al.* (2012)
Korea	1202	0.275[a]	0.088[a]	0.012[a]	1527	0[a]	0.036[a]	Kurose *et al.* (2012)
South Eastern								
Thailand	895	0.320[a]	0.040[a]	NA	242	0[a]	0.025[a]	Kurose *et al.* (2012)
Vietnam	165	0.264[a]	0.049[a]	NA	157	0[a]	0.022[a]	Kurose *et al.* (2012)
Southern								
India	1165	0.352[a]	0.037[a]	NA	583	0.044[a]	0.059[b]	Kurose *et al.* (2012)
Pakistan	68	0.272[a]	NA	NA	188	0.029[a]	0.114[a]	Kurose *et al.* (2012)
America								
African–American	108	0.250[a]	0[b]	NA	688	0.022[a]	0.018[a]	Hamdy *et al.* (2002); Kurose *et al.* (2012)
European–American	210	0.129[b]	0[b]	NA	100	0.080[b]	0.060[b]	Goldstein *et al.* (1997)

N, number of subjects; [a]$P < 0.05$, populations are significantly different from Tunisian population; [b]$P > 0.05$, populations are not significantly different from Tunisian population; NA, not available. The reported frequencies from our study are in bold.

Table 3. Allele (a) and genotype (b) frequencies of CYP2C9 in Tunisian subjects ($n = 258$).

(a)	dbSNP ID	SNP (variant allele)	*N* (frequency)	95% CI	HWE *P* value
	rs1799853 (430C>T)	CYP2C9*2 (T)	72 (0.139)	0.108–0.169	0.152
	rs1057910 (1075A>C)	CYP2C9*3 (C)	42 (0.081)	0.056–0.105	0.077

(b)	Phenotype	Genotype	*N* subjects	Frequency (%)	95% CI
	EM	CYP2C9*1/*1	159	61.6	55.5–67.6
	IM	CYP2C9*1/*2	50	19.4	14.4–24.3
		CYP2C9*1/*3	34	13.2	8.9–17.4
	PM	CYP2C9*2/*2	07	02.7	0.6–4.7
		CYP2C9*2/*3	08	03.1	0.9–5.2
		CYP2C9*3/*3	0	0	0

dbSNP ID, database single-nucleotide polymorphisms identifier; EM, extensive metabolizers; HWE, Hardy–Weinberg equation; IM, intermediate metabolizers; *N*, number; PM, poor metabolizers; SNP, single nucleotide polymorphism.

Table 4. Frequency of haplotypes inferred in Tunisian population ($n = 258$).

Haplotype	CYP2C19*17	CYP2C19*2	CYP2C9*2	CYP2C9*3	Frequency	Number of subjects
1	−	−	−	−	52.68	272
2	+	−	−	−	17.05	88
3	−	−	+	−	10.76	56
4	−	+	−	−	8.02	41
5	−	−	−	+	6.05	31
6	+	−	+	−	2.04	11
7	+	−	−	+	1.24	6

+, Allele is present; −, allele is absent.

populations (Pedersen *et al.* 2010). The authors reported that CYP2C19*17 exists in strong LD with the wild-type CYP2C9*1 and CYP2C8*1 in Danish, Faroese and Norwegian individuals, thus mediating rapid metabolism of CYP2C19 substrates and normal (mostly referred to as extensive) metabolism of CYP2C8 and CYP2C9 substrates (Pedersen *et al.* 2010). On the other hand, Suarez-Kurtz (2011) reported a haplotype containing CYP2C19*17 and CYP2C8*2 in populations of Brazilian individuals of African descent, which opposes CYP2C9*17 as a marker for extensive CYP2C8 substrate metabolism and concluded that further multiethnic CYP2C haplotype studies including CYP2C19*17 were warranted.

Conclusion

The definition of allele distribution pattern among populations represents a helpful support in the safety use of drugs for patients throughout the world. The allele frequency distributions for CYP2C19 and CYP2C9 variants among the Tunisian population are comparable to those among other European and Middle Eastern (including Bahraini and Kuwaiti), but they significantly differ from those among African and Asian populations. Moreover, we have identified a common haplotype in the Tunisian population carrying the CYP2C19*17, CYP2C9*1 alleles, thus representing a haplotype encoding efficient metabolism of drugs that are substrates for CYP2C enzymes. Our results give support to understand the ethnic diversity of the Tunisian population, and offer a preliminary basis for more rational use of drugs that are substrates for CYP2C9 and CYP2C19 in this population.

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