Saudi Pharmaceutical Journal 26 (2018) 651–655

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Distribution of *CYP2B6 516G/T* pharmacogenetically important polymorphism in the Ukrainian population

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ARTICLE INFO

Article history:
Received 5 December 2017
Accepted 13 February 2018
Available online 15 February 2018

Keywords:
CYP2B6 (516G/T) polymorphism
Ukraine
Population

ABSTRACT

The CYP2B6 is one of the members of the cytochrome P450 superfamily. This enzyme metabolizes a number of currently prescribed drugs and different compounds. In light of clinical significance of the *CYP2B6*∗*6* variant of the *CYP2B6* gene, the aim of this study was to investigate the distribution of one of the gene polymorphisms, namely, the *516G/T* in the Ukrainian population.

The study cohort consisted of 102 healthy Ukrainian adults (48 males, 54 females). Genotyping of the *CYP2B6* (rs3745274) polymorphism in the study subjects was carried out using a polymerase chain reaction.

The following distribution of *516G/T CYP2B6* genotypes in the Ukrainian cohort was identified: *GG* – in 56%, *GT* – in 37% and *TT* – in 7%. The *516G/T* allele frequency of the *CYP2B6* gene in population was p_G = 0.75 and q_T = 0.25, respectively. The population-based sequences were analyzed by the Hardy-Weinberg method.

The genetic polymorphism revealed in the Ukrainian population suggests the *516G/T* polymorphism of the *CYP2B6* genetic testing when prescribing the drugs that are substrates of this gene.

1. Introduction

The CYP2B6 is one of the members of the cytochrome P450 superfamily. This enzyme metabolizes a number of currently prescribed drugs and different compounds, such as nicotine (Yamazaki et al., 1999), cyclophosphamide, bupropion, efavirenz (Scibona et al., 2015) and ketamine (Li et al., 2015).

The CYP2B6 is a high polymorphic isoenzyme that is encoded by the gene located in the chromosome 19. Numerous allelic forms are responsible for the proteins with varying degrees of enzyme activity. In particular, the *CYP2B6*∗*6* allele variant of the *CYP2B6* gene is associated with its decreased activity. A typical example of the single nucleotide polymorphism of the *CYP2B6* with decreased enzymatic activity is the variant *516G/T* relating to the *CYP2B6*∗*6* allele. Phenotypically, the *TT* homozygotes are poor metabolizers (highly toxic drug), the *GT* heterozygotes are characterized by intermediate activity of the CYP2B6 enzyme (therapeutic dose of the drug is recommended), and the *GG* homozygotes are rapid metabolizers (increased dose of the drug is recommended) (Scibona et al., 2015).

Numerous studies point to the presence of clinical associations of the *CYP2B6* variants. Thus, in poor metabolizers of the CYP2B6, the clinical consequences of taking drugs metabolized by the corresponding enzyme can be, for example, a delayed activation of cyclophosphamide and an increased level of efavirenz, which in turn can have a toxic effect on the central nervous system (Scibona et al., 2015). In particular, one study in China showed pharmacokinetic differences in HIV-infected patients with different genotypes of *CYP2B6 516G/T* who underwent antiviral therapy with efavirenz. The accumulation of efavirenz might occur over time, leading to neurotoxicity in subjects with *TT* and *GT* genotypes (To et al., 2009). It was shown that the *CYP2B6*∗*6* allele was associated

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2018.02.027
1319-0164/

Table 1
Distribution of individuals by their birthplaces.

Birthplace	Males, *n* (%)	Females *n* (%)	Total, *n* (%)
Kharkiv and Kharkiv Oblast, Ukraine	10 (21.7)	13 (27.7)	23 (24.7)
Lugansk and Lugansk Oblast, Ukraine	8 (17.4)	12 (25.5)	20 (21.5)
Donetsk and Donetsk Oblast, Ukraine	13 (28.3)	5 (10.6)	18 (19.4)
Other Oblasts	15 (32.6)	17 (36.2)	32 (34.4)
Total	46 (100)	47 (100)	93 (100)

Note *: $\chi^2 = 4.9$, $v = 3$, $p > 0.05$. Other regions included the following: Poltava and Poltava Oblast, Chernihiv and Chernihiv Oblast, Sumy and Sumy Oblast, Dnipro and Dnipro Oblast, Vinnytsia and Vinnytsia Oblast, Kropyvnytskyi and Kropyvnytskyi Oblast, Lviv and Lviv Oblast, Volhynia and Volhynia Obalst, Odessa and Odessa Oblast, former USSR republics.

with a significantly reduced metabolism of ketamine in vitro, and anesthesia with this drug was complicated due to interindividual differences. Thus, in a study conducted by Australian scientists in patients with chronic pain using ketamine and being slow metabolizers, there was a reduced clearance and higher plasma ketamine concentrations leading to increased drug-related side effects (Li et al., 2015). The study conducted in Israel showed the role of the *CYP2B6* gene polymorphism in the methadone treatment of opioids, in particular, heroin addicted patients. The *CYP2B6∗6 516G/T* (rs3745274) allele was associated with a slow metabolism of methadone. The average dose of Methadone required for the homozygous for the altered *516G/T* allele patients was significantly lower than the dose required for heterozygotes and homozygous wild individuals (Levran et al., 2013).

There is some evidence indicating that in some cases the role of the *CYP2B6* genotype can be smoothed, and the toxic effects of the drugs may be expressed by either fast or slow metabolizers. Thus, potentially toxic concentrations of the main metabolite efavirenz (8-hydroxy-efavirenz) may be often observed in the cerebrospinal fluid, regardless of the *CYP2B6* genotype, especially in individuals with breach of the blood-brain barrier (Nightingale et al., 2016).

In light of clinical significance of the *CYP2B6∗6* variant of the *CYP2B6* gene, the aim of this study was to investigate the distribution of one of the gene polymorphysims, namely, the *516G/T* in the Ukrainian population.

2. Subjects and methods

To investigate the distribution of the *516G/T CYP2B6* polymorphism a study cohort consisting of Ukrainian adults was formed. In total, the genetic material was collected from 102 subjects (48 males, 54 females), who were not related to each other. The population of Ukraine is mainly represented by the Ukrainians and Russians (Atramentova and Filiptsova, 1998, 1999; Atramentova et al., 2000) as previously shown in our studies.

The individuals from the current study indicated their birthplaces as presented in Table 1. For the further analysis only known information (n = 93) was used, because some participants did not present this data.

The ethnicity of subjects, participated in the study, has been evaluated by the parental and grandparental ethnic origin separately for males and females. This was done due to the fact, that ethnicity in Ukraine is often associated with a citizenship. While in the former USSR ethnicity was included in the passport, in the modern Ukraine this position is not present there. This is the reason why young people confuse their ethnicity and citizenship but information, provided by older people, is much more reliable. It can be observed, that majority of subjects under study had the closest relatives, which were Ukrainians and Russians (Table 2). Some ethnical minorities were the following: Crimean Tatars, Belarusians, Tatars, Greeks, Germans, Bulgarians, Yakuts, Poles, Slovenians. Likewise, the calculations were done only for known

Table 2
Ethnic composition of parents and grandparents of subjects under study.

Ethnicity by maternal line*				Ethnicity by paternal line**			
	Males, *n* (%)	Females, *n* (%)	Total *n (%)*		Males, *n* (%)	Females, *n* (%)	Total *n (%)*
Mother's ethnicity is*				Father's ethnicity is**			
Ukrainian	41 (85.4)	41 (82)	82 (83.7)	Ukrainian	35 (73)	36 (72)	71 (72.5)
Russian	5 (10.4)	9 (18)	14 (14.3)	Russian	11 (23)	14 (28)	25 (25.5)
Other ethnicities	2 (4.2)	0	2 (2)	Other populations	2 (4)	0	2 (2)
Total	48 (1 0 0)	50 (1 0 0)	98 (1 0 0)	Total	48 (1 0 0)	50 (1 0 0)	98 (1 0 0)
Maternal grandmother's ethnicity is***				Paternal grandmother's ethnicity is****			
Ukrainian	35 (81.4)	33 (70.2)	68 (75.5)	Ukrainian	29 66	30 65.2	59 (65.6)
Russian	4 (9.3)	12 (25.5)	16 (17.8)	Russian	11 25	13 28.3	24 (26.7)
Other populations	4 (9.3)	2 (4.3)	6 (6.7)	Other populations	4 9	3 6.5	7 (7.7)
Total	43 (1 0 0)	47 (1 0 0)	90,100	Total	44,100	46,100	90 (1 0 0)
Maternal grandfather's ethnicity is*****				Paternal grandfather's ethnicity is******			
Ukrainian	29 (69)	26 (55.3)	55 (61.8)	Ukrainian	29 (67.4)	27 (61.4)	56 (64.4)
Russian	10 (23.8)	20 (42.6)	30 (33.7)	Russian	9 (21)	14 (31.8)	23 (26.4)
Other populations	3 (7.2)	1 (2.1)	4 (4.5)	Other populations	5 (11.6)	3 (6.8)	8 (9.2)
Total	42 (1 0 0)	47 (1 0 0)	89 (1 0 0)	Total	43 (1 0 0)	44 (1 0 0)	87 (1 0 0)

Notes. For * $\chi^2 = 3.1$, $v = 2$, $p > 0.5$; for ** $\chi^2 = 2.3$, $v = 2$ $p > 0.5$; for *** $\chi^2 = 4.6$, $v = 2$, $p > 0.5$; for **** $\chi^2 = 0.3$, $v = 2$, $p > 0.5$; for ***** $\chi^2 = 4.2$, $v = 2$ $p > 0.5$, for ****** $\chi^2 = 1.6$, $v = 2$, $p > 0.5$.

data, so that is why the actual numbers of relatives taken into account can be different.

The buccal epithelium sampling was taken. Genotyping of the *CYP2B6* (rs3745274) polymorphism in the study subjects was carried out using a polymerase chain reaction.

DNA was isolated from the buccal epithelium samples of each subject using the ion-exchange resin Chelex-100 (Walsh et al., 1991). The allelic state of the *CYP2B6*6* gene was determined by allelic discrimination with *516G/T* (rs3745274) according to the procedure (Masebe et al., 2012). Amplification was carried out on a thermocycler "Terzik" (DNA-Technology, Russia).

The AGGTGACAGCCTGATGTTCC (forward) and TTTCTCGTGTGTTCTGGGTG (reverse) oligonucleotide primers (Masebe et al., 2012) were used to amplify the fragment of the *CYP2B6* gene containing the polymorphic site (*516G/T*). Restriction of the amplification products was carried out with BseNI endonuclease (MBI Fermentas, Lithuania). The amplification products were analyzed with the electrophoresis in a 2% agarose gel. As a molecular weight marker pUC19 DNA hydrolysed with MspI endonuclease (MBI Fermentas, Lithuania) was used. The resulting PRA products were visualized by electrophoresis on a 2% agarose gel. The restriction fragment of 289 bp corresponded to the uncut product (*TT*) under the *516G/T* variant of the *CYP2B6* gene, and two fragments of 196 and 93 bp to the wild type (*GG*). The presence of all three bands on the electrophoretogram indicated a heterozygous product (*GT*) (Masebe et al., 2012).

Allele and genotype frequencies (p and q) were estimated by gene counting:

$$p_G = \frac{2GG + GT}{2N} \text{ and } q_T = \frac{2TT + GT}{2N}.$$

where N - number of study subjects.

Genetic diversity based on allele frequencies was assessed using the χ^2 criterion. A significance level $p \leq 0.05$ was considered statistically significant.

3. Results and discussion

Fig. 1 shows the results of electrophoresis in a 2% agarose gel amplified in PCR and human DNA was digested with BseNI hydrolyzed endonuclease.

Genotyping procedure for the *516G/T* polymorphism of the *CYP2B6* gene showed that in the study cohort the number of poor (*TT*, 7 out of 102) metabolizers was the lowest, while the number of rapid (*GG*, 57 out of 102) ones was the highest. In general, in the studied population, the percentage distribution of the genotypes was as follows: *GG* – in 56%, *GT* – in 37% and *TT* – in 7% (Table 3).

Table 3
Distribution of the *516G/T* polymorphism of the ***CYP2B6* gene.**

	Males, *n*	Females, *n*	Total, *N* (%)
GG	27	30	57 (56)
GT	16	22	38 (37)
TT	5	2	7 (7)
Statistics: $\chi^2 = 0.656$, df = 2, p > 0.05			

Note. χ^2 – Pearson's criterion, *df* – degree of freedom, *p* – significance level.

Table 4
Frequency of the *G* and *T* allele of the ***CYP2B6* gene** (*516G/T* polymorphism).

	Alleles	
	G	*T*
Males	0.73	0.27
Females	0.76	0.24
Total	0.75	0.25

Table 5
Genotype frequencies of the *516G/T* polymorphism of the ***CYP2B6*** gene.

	Genotypes		
	GG	*GT*	*TT*
Males	0.54	0.39	0.07
Females	0.58	0.36	0.06
Total	0.56	0.38	0.06

Table 6
The observed and expected genotype frequencies of the *516G/T* polymorphism of the ***CYP2B6*** gene.

	Expected genotype frequencies	Observed genotype frequencies
GG	56	57
GT	37	38
TT	6	7
Statistics: $\chi^2 = 0.054$, *df* = 2, *p* > 0.05		

Note. All designations are the same, as in Table 3.

The *516G/T* allele frequency of the *CYP2B6* gene in population was $p_G = 0.75$ and $q_T = 0.25$. The population-based sequences were analyzed by the Hardy-Weinberg method.

We calculated the *G* and *T* allele frequencies for males and females on an individual basis, as well as, the observed and expected frequencies of the corresponding alleles: p_G – 0.75 and q_T – 0.25, respectively (Table 4).

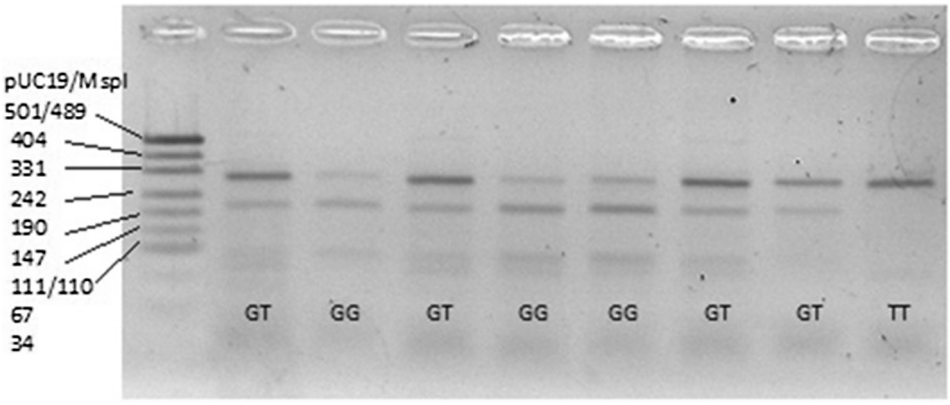

Fig. 1. Electrophoresis in a 2% agarose gel amplified in PCR and human DNA was digested with BseNI hydrolyzed endonuclease: M-pUC19/MspI marker, 1-8 – DNA of the study subjects.

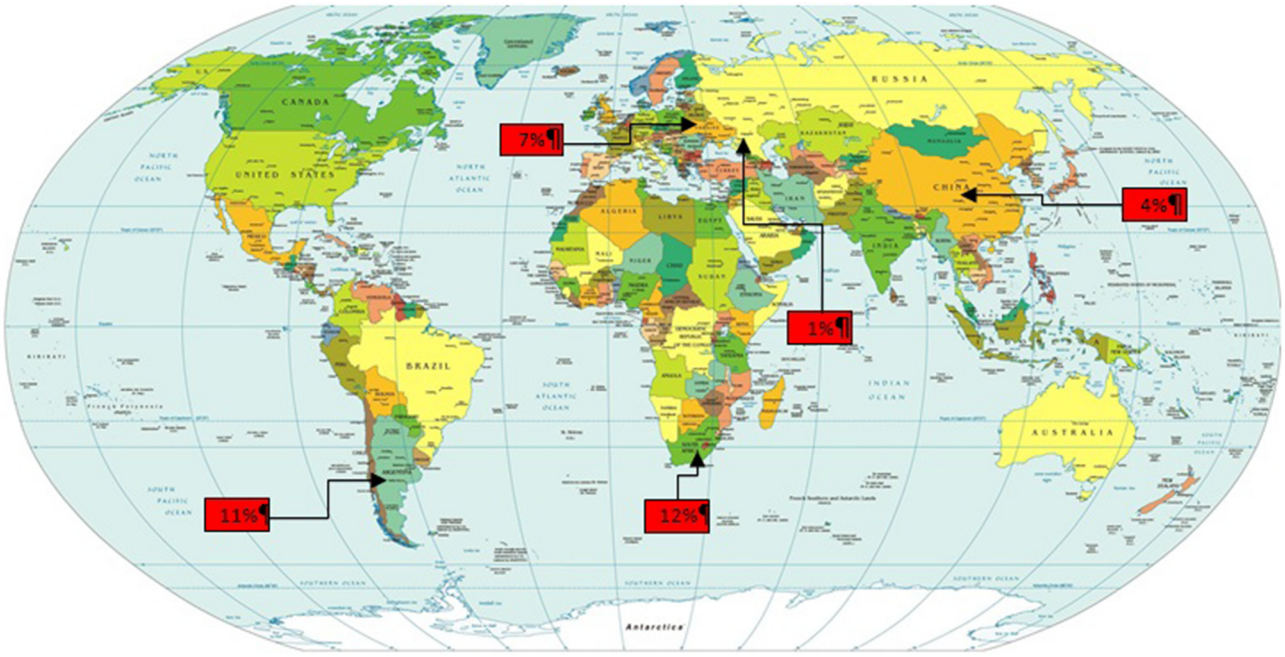

Fig. 2. Frequency of poor metabolizers, *TT* homozygotes under the *516G/T* polymorphism of the *CYP2B6* gene (from the data in the literature (see references in the text) and own research).

Allele frequencies, expected genotypes and equilibrium of alleles in the population were analyzed by the Hardy–Weinberg method (Table 5).

The observed and expected genotype and allele frequencies did not show statistically significant differences compared to those expected under the Hardy-Weinberg Equilibrium (Table 6). This allows us to make a conclusion about the *516G/T* polymorphism of the *CYP2B6* gene in the studied Ukrainian population.

The frequencies of these alleles were studied in a number of populations, and, as the study showed, the world population is diverse. Analysis of population-based sequences of the allele *G* with 102 subjects from Argentina revealed a frequency of 71%, and the allele T – a frequency of 29%, respectively. The frequencies of the corresponding genotypes were distributed as follows: *GG* – in 52%, *GT* – in 37% and *TT* – in 11% of the study subjects. Sexual differences in the distribution of genotypes were not observed. Genotyping should be used as an additional tool for personalized medicine in connection with the high prevalence of the *TT* genotype in the studied population (Scibona et al., 2015). The study conducted in China determined the frequency of *CYP2B6 516G/T* mutation in 79 HIV infected patients. *GG* genotype in the studied population was determined in 42 (53%), in 34 – *GT* (43%) and in 3 – *TT* (4%). The population frequency of *T* allele comprised 0.25 (To et al., 2009). Analysis of population-based sequences of *516G/T* polymorphism of the *CYP2B6* gene with subjects from province of Limpopo in South Africa showed a relatively high frequency of slow metabolizers. 12% of 199 HIV-infected individuals had the homozygous *TT* genotype, 78% – the *GG* genotype, and 10% – the heterozygote *GT* genotype, respectively (Masebe et al., 2012).

The results of a single study of *516G/T* population-based polymorphism in the Slavic population are known. For example, in the study cohort consisted of 354 Rostov-on-Don residents, 283 volunteers (80%) were rapid (*GG*), 68 (19%) – intermediate (*GT*) and 3 (1%) – poor (*TT*) metabolizers (Махарин, 2012).

Taking into account the above-mentioned data, it can be found out that the studied gene frequencies and, respectively, the frequencies of different genotypes indicate the presence of interindividual differences in the *CYP2B6 516G/T* mutation (Fig. 2). Nevertheless this polymorphism has an important clinical significance, generally people in Ukraine are far from understanding importance of genotyping, mostly due to the subjective assessment of high price for such genetic tests, as our previous research has demonstrated (Filiptsova et al., 2017).

4. Conclusions

1. This study determined the following genotype distribution under the *516G/T* polymorphism of the *CYP2B6* gene in the Ukrainian population: *GG* – in 56%, *GT* – in 37% and *TT* – in 7%.
2. Population-based frequencies of the *516G/T* allele of the *CYP2B6* gene comprised $p_G = 0.75$ and $q_T = 0.25$.
3. The observed and expected genotype and allele frequencies did not show statistically significant differences compared to those expected under the Hardy–Weinberg Equilibrium.
4. Genetic polymorphism revealed in the Ukrainian population is the basis for recommending genetic testing for the *516G/T* polymorphism for therapy optimization with drugs that are substrates of the *CYP2B6* gene.

Acknowledgments

The study was carried out funded by the Ministry of Education and Science of Ukraine “Biological Challenges and Threats due to Migrations and Invasions: a Population-Genetic Approach” (2017).

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