Original article

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

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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

1. Introduction

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CYP2B6 in particular, heroin addicted patients. The gene polymorphism in the methadone treatment of opioids, CYP2B6 et al., 2015). The study conducted in Israel showed the role of the concentrations leading to increased drug-related side effects (Li alizers, there was a reduced clearance and higher plasma ketamine differences. Thus, in a study conducted by Australian scientists in 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 polymorphisms, 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

| Birthplace                  | Males, n (%) | Females, n (%) | Total, n (%) |
|-----------------------------|--------------|----------------|--------------|
| Kharkiv and Kharkiv Oblast, Ukraine | 10 (21.7)    | 13 (27.7)      | 23 (49.4)    |
| Lugansk and Lugansk Oblast, Ukraine | 8 (17.4)     | 12 (25.5)      | 20 (41.4)    |
| Donetsk and Donetsk Oblast, Ukraine | 13 (28.3)    | 5 (10.6)       | 18 (36.6)    |
| Other Oblasts               | 15 (32.6)    | 17 (36.2)      | 32 (64.4)    |
| 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, Dniprop, Dniprop Oblast, Vinnytsya and Vinnytsya Oblast, Kropyvnytskyi and Kropyvnytskyi Oblast, Liviv and Liviv Oblast, Volhynia and Volhynia Oblast, Odessa and Odessa Oblast, former USSR republics.

| Ethnicity by maternal line | Males, n (%) | Females, n (%) | Total n (%) |
|---------------------------|--------------|----------------|-------------|
| Mother's ethnicity is...   |              |                |             |
| Ukrainian                 | 41 (85.4)    | 41 (83.7)      | 82 (100)    |
| Russian                   | 5 (10.4)     | 9 (14.3)       | 14 (25)     |
| Other ethnicities         | 2 (4.2)      | 2 (2)          | 4 (2)       |
| Total                     | 48 (100)     | 50 (100)       | 98 (100)    |
| Maternal grandmother's ethnicity is... | | | |
| Ukrainian                 | 35 (81.4)    | 33 (75.5)      | 68 (100)    |
| Russian                   | 4 (9.3)      | 12 (25.5)      | 16 (17.6)   |
| Other populations         | 4 (9.3)      | 2 (4.3)        | 6 (6.7)     |
| Total                     | 43 (100)     | 47 (100)       | 90,100      |

| Ethnicity by paternal line | Males, n (%) | Females, n (%) | Total n (%) |
|---------------------------|--------------|----------------|-------------|
| Father's ethnicity is...  |              |                |             |
| Ukrainian                 | 35 (73)      | 36 (72)        | 71 (100)    |
| Russian                   | 11 (23)      | 14 (28)        | 25 (37.5)   |
| Other populations         | 2 (4)        | 0 (2)          | 2 (3)       |
| Total                     | 48 (100)     | 50 (100)       | 98 (100)    |
| Paternal grandmother's ethnicity is... | | | |
| Ukrainian                 | 29 (66)      | 30 (65.2)      | 59 (100)    |
| Russian                   | 11 (25)      | 13 (28.3)      | 24 (38)     |
| Other populations         | 4 (9)        | 3 (6.5)        | 7 (7)       |
| Total                     | 44,100       | 46,100         | 90,200      |

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:

\[ p_G = \frac{2GG + GT}{2N} \]
\[ q_T = \frac{2TT + GT}{2N} \]

where \( N \) - number of study subjects.

Genetic diversity based on allele frequencies was assessed using the \( \chi^2 \) 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**

| 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. \( \chi^2 \) – 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.

| GG       | GT       | TT  |
|----------|----------|-----|
| Males    | 0.54     | 0.39| 0.07|
| Females  | 0.38     | 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](image_url)

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.
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 (Maxapov, 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 (Filipstsova 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 pG = 0.75 and qT = 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.

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