Allele Frequencies of 163А/С Polymorphism of the CYP1A2 gene in the Selected Ukrainian Population

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ABSTRACT

Background: CYP1A2 is a very important gene for a potential genotyping. The objective of this work was to study the population frequencies of the corresponding 163А/C single nucleotide polymorphism of the CYP1A2 gene in the sampling of the Ukrainian population.

Methods: Total sampling of genetic material (buccal epithelium) was collected in 102 subjects (48 males, 54 females) not related to each other. Genotype test of the participants of the study for CYP1A2 polymorphism (rs762551) was performed using the polymerase chain reaction. In accordance with the findings of genotyping the allele frequencies ($p_A$ and $q_C$) were calculated.

Results: The following distribution of genotypes in a sampling of the Ukrainian population under the 163А/C polymorphism of the CYP1A2 gene: AA - 36%, AC - 49% and CC - 15% in the subjects was revealed. The population allele frequencies of the 163А/C polymorphism of the CYP1A2 gene totaled $p_A = 0.6$ and $q_C = 0.4$. The population structure of the individuals does not deviate from the Hardy-Weinberg equilibrium, as may be seen from the lack of difference between the theoretically expected and the observed frequencies of the three genotypes.

Conclusion: The genetic polymorphism found in the Ukrainian population is the basis for the recommendation of the genetic testing of 163А/C polymorphism of the CYP1A2 gene in the prescribing drugs - substrates of this gene.

Key Words: CYP1A2 (163А/C polymorphism), population distribution, Ukraine.

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ÖZET

Amaç: CYP1A2, potansiyel bir genotipleme için çok önemli bir gendir. Bu çalışmanın amacı, Ukrayna nüfusunun örneklemesinde CYP1A2 geninin 163А/C tek nükleotit polimorfizminin populasyon frekanslarını incelemektir.

Yöntem: Birbiriyle akraba olmayan 102 denekten (48 erkek, 54 kadın) genetik materyal (bukkal epitel) toplandı. CYP1A2 polimorfizmi (rs762551) için çalışma katılımcılarının genotip testi, polimeraz zincir reaksiyonu kullanılarak gerçekleştirilmüş. Genotipleme bulgularına göre allele frekansları ($p_A$ ve $q_C$) hesaplanmıştır.

Bulgular: CYP1A2 geninin 163А/C polimorfizmi Ukrayna populasyonunun örneklemesinde aşağıdaki genotip dağılımı göstermiştir: % 36 AA, % 49 AC ve % 15 CC. CYP1A2 geninin 163А/C polimorfizminin populasyon allele frekansları, $p_A = 0.6$ ve $q_C = 0.4$ olmuştur. Bireylerin populasyon yapısı, teorik olarak beklenen ve gözlenen üç genotipin sıraları arasındaki farkın eksikliğinde görülebileceği gibi Hardy-Weinberg dengesinden sapmaz.

Sonuç: Ukraynalı populasyonunda bulunan genetik polimorfizm, CYP1A2 geninin substratları olarak reçete edilen ilaçlarda bu geninin 163А/C polimorfizminin genetik testinin önerilmesine temel olusturulmaktadır.

Anahtar Sözcükler: CYP1A2 (163А/C polimorfizmi), nüfus dağılımı, Ukrayna.

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INTRODUCTION

The CYP1A2 gene is located in chromosome 15 and composed of 7 exons and 6 introns (1). CYP1A2 is one of the major liver enzymes, which is involved in the metabolism of the variety of drugs, widespread suspected carcinogens and endogenous compounds. As the cytochrome is expressed in the liver, it can metabolize the compounds without additional induction. Among the drugs, which undergo a substantial (over 30%) metabolic transformation under its influence, are, in particular, caffeine, theophylline, tacrine, melatonin, verapamil, clozapine, olanzapine, aminopyrine, propranolol, etc. The drugs, that to a greater or lesser (30 to 10%) extent are exposed to the action of CYP1A2, include acetaminophen, lidocaine, imipramine, antipyrin, etc. Examples of the suspected carcinogens activated by CYP1A2 are benzoquinone and aflatoxin B1, and the examples of the endogenous compounds are steroids. CYP1A2 has a number of inducers and suppressors, which can temporarily alter its genetically determined activity. Some of allele forms of CYP1A2 are particularly sensitive to the effect of the respective inducers (2).

So far, there are more than 30 variants of CYP1A2 allelic forms. In particular, during the study of DNA samples taken from Japanese individuals, the most common single nucleotide polymorphism was 163A/C (rs762551), which is defined as an allele CYP1A2*1F (3). In one study it was offered to define the allele as the C allele of the wild-type, and the A allele — as a mutant variant (4). It is also known, that from the several variants of the gene CYP1A2 allele CYP1A2*1F is marked by a particularly high inducibility (5). CYP1A2*1F is responsible for the shape of the enzyme with poor metabolic activity (2). For example, earlier studies have shown, that smoking increased the activity of the enzyme CYP1A2. On this inducing influence an increased in the metabolic ratio of caffeine was clearly indicated in smokers. In the males – inveterate smokers, the enzyme activity was also correlated with the metabolism of acetylmophine, which is partly manifested in the glucuronidation of the compound (6).

The study is known, that examined the effect of the CYP1A2 gene polymorphism on early aging due to industrial hazards. The results of one study pointed at a higher level of DNA damage in the cells and, consequently, their premature deterioration and aging, that had a mutant form of CYP1A2 and were exposed to harmful compounds (7).

As the rs762551 genetic polymorphism under the gene, encoding CYP1A2, affects the inducibility of the expression of CYP1A2 and may be associated with different types of cancer. One of the meta-analyses, that included 19 studies with a "case-control" design, illustrated the probable association of CYP1A2 gene and predisposition to chronic obstructive pulmonary disease (10). A similar study was conducted in Bashkortostan, but the relationship between the polymorphism of the CYP1A2 gene and predisposition to chronic obstructive pulmonary disease was not detected (11).

At the same time the study of treatment efficacy with betaxolol, a synthetic antihypertensive drug from the group of β-blockers, has shown that the therapeutic effect, to a greater extent, had been achieved for the poor metabolizers – subjects with CC genotype of 163A/C variant of the CYP1A2 gene. The authors of the study attributed this to the fact, that the metabolism of the drug had been reduced, and its therapeutic effect on the patients was longer. The observed effect was shown in the Russian population (12).

It was also shown, that in poor CC metabolizers clearance of teriflunomide, which is the active metabolite of the anti-rheumatic drug leflunomide, was slightly lower, than in the extensive (AA) and intermediate (AC) metabolizers. However, the noted phenomenon was only a trend (13).

CYP1A2 is a very important gene for a potential genotyping due to all described above and it was not yet been studied by any polymorphism in the population of Ukraine. In our previous research we have demonstrated that understanding of pharmacogenetics is different in males and females, but it is highly desirable it be increasing (14). Even in this situation some part of the Ukrainians is ready to pay for genetic tests which are part of personalized medicine (15). The objective of this work was to study the population frequencies of the corresponding 163A/C single nucleotide polymorphism of the CYP1A2 gene in the sampling of the Ukrainian population.

MATERIALS and METHODS

To study the population distribution of the 163A/C polymorphism of the CYP1A2 gene a sampling consisting of the Ukrainian individuals was formed. It should be noted that ethnicities of people living in the area of the modern Ukraine are predominantly Ukrainians and Russians with different proportions in the different locations of the country (16-18). Total sampling of genetic material (buccal epithelium) was collected in 102 subjects (48 males, 54 females), who were not related to each other. Participants were predominantly healthy students of National University of Pharmacy, aged 17 to 25. Material was collected in accordance with ethical standards of work under Helsinki Declaration (World Medical Association Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects). Genotype test of the participants of the study for CYP1A2 polymorphism (rs762551) was performed using the polymerase chain reaction (19).

The DNA was isolated from the test samples of buccal epithelium using the ion exchange resin Chelex-100 (20). Determination of the allelic states of the CYP1A2*1F gene under the single nucleotide replacement of CYP1A2 (rs762551) was carried out according to the standard methods (4). The amplification was performed on the "Tertsik" thermocycler (amplifier) (DNA-Technology, Russia).

For the fragment amplification of the CYP1A2 gene, which contained the polymorphic site (163A/C) Oligonucleotide primers were used: the forward CCGAAGTGGAAACTGA and the reverse GGGTGGAGATGGAGACATT (4). The restriction of the amplification products was performed using the Apal endonuclease (MBI Ferments, Lithuania). The restriction of amplification products was analyzed with electrophoresis in 2% agarose gel. As the marker of molecular weight the DNA pUC19 was used, hydrolyzed with the MspI endonuclease (MBI Ferments, Lithuania). The visualization of the amplification and restriction products were carried out by the staining with ethidium bromide and photographing at the transilluminator under the UV light. The restriction fragment of 243 bp size corresponded to A allele under the variant 163A/C of the CYP1A2 gene, and two restriction fragments of 119 and 124 bp size corresponded to the C allele. The presence of all three bands at the electrophoregram indicated at the heterozygous AC genotype (4).

In accordance with the findings of genotyping the allele frequencies (p and q) were calculated:

\[ P_A = \frac{2 \cdot AA + AC}{2N} \quad \text{and} \quad q_C = \frac{2 \cdot CC + AC}{2N} \]

where \( N \) is a number of subjects.

The testing of the genotypes distribution on Hardy-Weinberg equilibrium using the criterion \( \chi^2 \) was performed. The statistical hypothesis testing at \( p \leq 0.05 \) level of significance was conducted.
RESULTS

Figure 1 shows the findings of electrophoretic fractionation in 2% agarose gel of human DNA amplified in PCR and hydrolyzed by endonuclease ApaI. Genotype test of the subjects for the 163A/C polymorphism of the CYP1A2 gene found, that in the studied sampling the fewest were poor (CC, 15 of 102), and the most were intermediate (AC, 50 of 102) metabolizers. In general, in the population sampling the percentage distribution of the genotypes was as follows: AA — in 36%, AC — in 49% and CC — in 15% subjects (Table 1).

Figure 1. The electrophoretic fractionation in 2% agarose gel of the human DNA amplified PCR and hydrolyzed by endonuclease ApaI: first marker-band- pUC19/Mspl, 2-13 marker-bands - DNA of the subjects.

Table 1. The distribution of genotypes of 163A/C polymorphism of CYP1A2 gene

| Genotypes | Males, n | Females, n | Total, N (%) |
|-----------|----------|------------|--------------|
| AA        | 19       | 18         | 37 (36%)     |
| AC        | 21       | 29         | 50 (49%)     |
| CC        | 8        | 7          | 15 (15%)     |

Statistics: $\chi^2 = 1.024$, df = 2, $p > 0.05$.

Note. $\chi^2$ – Pearson criterion, df – degree of freedom, $p$ – significance level.

Separately for males and females the allelic frequencies $A$ and $C$ were calculated. The weighted average frequencies of corresponding alleles which totaled: $p_A = 0.6$ and $q_C = 0.4$, respectively were also calculated (Table 2).

Table 2. The allelic frequency of $A$ and $C$ of CYP1A2 gene (163A/C polymorphism)

| Alleles | $A$ | $C$ |
|---------|-----|-----|
| Males   | 0.61| 0.39|
| Females | 0.6 | 0.4 |
| Total   | 0.6 | 0.4 |

According to the allele frequencies, on the basis of Hardy-Weinberg proportions were calculated the frequencies of the respective genotypes (Table 3).

Table 3. The genotype frequencies under the 163A/C polymorphism of the CYP1A2 gene

| Genotypes | AA   | AC   | CC   |
|-----------|------|------|------|
| Males     | 0.37 | 0.48 | 0.15 |
| Females   | 0.36 | 0.48 | 0.16 |
| Total     | 0.36 | 0.48 | 0.16 |

The theoretically expected genotype frequencies calculated from the Hardy-Weinberg equilibrium was not significantly different from the observed ones (Table 4).

Table 4. The theoretically expected population genotype frequencies of the polymorphism 163A/C of the gene CYP1A2

| Genotypes | Theoretically expected genotype frequencies | Observed genotype frequencies |
|-----------|--------------------------------------------|------------------------------|
| AA        | 37                                         | 37                           |
| AC        | 49                                         | 50                           |
| CC        | 16                                         | 15                           |

Statistics: $\chi^2 = 0.042$, df = 2, $p > 0.05$.

Note. The designations are the same as for Table 1.
The frequencies of these alleles were studied in several populations. The studies have shown that the world’s population is variable.

The study the 163A/C polymorphism distribution of the CYP1A2 gene in 159 healthy Japanese individuals found, that CC homozygotes occurred at a frequency of 16.4%, homozygotes AA – 39% and heterozygotes AC – 44.6% (4). Among the 71 patients with rheumatoid arthritis at the age from 27 to 82, residents of Central Europe and being treated in the clinic of Slovenia CC genotype was present in 5.6%, AC in 56.3% and AA in 38% of the subjects, respectively. More detailed information about the origin of the subjects was not available (13). Among the healthy Turkish population (n = 101) and the patients with chronic obstructive pulmonary disease, the subjects with CC genotype occurred at a frequency of 6.9% and 11%, AC – 39.6% and 58%, and AC – 53.3% and 31%, respectively.

The respective allelic frequencies in healthy people were $p_А$ – 0.73 and $q_С$ – 0.27, and in patients – $p_А$ – 0.6 and $q_С$ – 0.4 (10).

The frequencies of different genotypes of the 163A/C polymorphism of the CYP1A2 gene in other Slavic populations can be assessed by the study conducted by Russian scientists. In particular, there is available information on the genotypes distribution in a sampling of the patients with cardiovascular diseases – hypertension and atrial fibrillation, with a total of 81 people. Thus, in the various subgroups separated depending on objectives of the study, the frequency of poor CC metabolizers was 8% and 27%. The extensive (AA) and intermediate (AC) metabolizers were 68%/64% and 24%/29%, respectively. The allele frequencies in different subgroups varied from 0.59 to 0.8, and C allele – from 0.2 to 0.41, respectively (12).

Thus, the data provided allowed to make a suggestion that the studied gene frequencies and, respectively, the frequencies of different genotypes, point at the presence of intra-population differences in the 163A/C polymorphism of the CYP1A2 gene (Figure 2), yet the small sample size should be defined as limitation of our study.

**Figures**: Figure 2. The frequency of poor metabolizers and CC homozygotes under the 163A/C polymorphism of the CYP1A2 gene (according to the references (see links in the text) and according to the original survey).

**CONCLUSIONS**

The following distribution of genotypes in a sampling of the Ukrainian population under the 163A/C polymorphism of the CYP1A2 gene: AA – 36%, AC – 49% and CC – 15% in the subjects was revealed. The population allele frequencies of the 163A/C polymorphism of the CYP1A2 gene totaled $p_А$ = 0.6 and $q_С$ = 0.4. The population structure of the individuals does not deviate from the Hardy-Weinberg equilibrium, as may be observed from the lack of difference between the theoretically expected and the frequencies of the three genotypes. The genetic polymorphism found in the Ukrainian population is the basis for the recommendation of the genetic testing of 163A/C polymorphism of the CYP1A2 gene in the prescribing drugs that are the substrates of this gene.

**Conflict of interest**

No conflict of interest was declared by the authors.

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