THE STUDY OF CYP1A2 (rs762551) POLYMORPHISM IN THE UKRAINIAN POPULATION SAMPLE

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Introduction. Cytochrome P450 1A2 (CYP1A2) is a member of the cytochrome P450 family, which participates in the oxidation of many compounds of endogenous and exogenous origin.

The compounds of endogenous origin can be such like fatty acids, steroids, prostaglandins, bile acids etc. Medicines, pesticides, poisons, carcinogens, mutagens are compounds of exogenous origin, otherwise known as xenobiotics. Cytochrome P450 1A2 (CYP1A2) participates in the metabolism of various drugs: theophylline, caffeine, clozapine etc. CYP1A2 plays a role also in the metabolism of caffeine contained in coffee.

It has been established that the carriers of the genotype AA are "rapid" metabolizers, and those who have AC or CC genotype are "rapid" metabolizers.

In this regard, for example, the same amount of caffeine will have a stimulating effect in poor metabolizers CYP1A2 compared with rapid CYP1A2 metabolizers. The prevalence of mutations (rs762551) in the European population is 30-50%.

Aim. The aim of the study was to study gene distribution of specific mutation (single nucleotide polymorphism) of CYP1A2 (rs762551) in Ukrainian population.

Materials and methods. 100 people, who do not share common genes, participated in the study. All volunteers were residents of Ukraine, and were ethnic Ukrainians and Russians. The biological material for study was buccal epithelium.

Participants genotyping on CYP1A2 (rs762551) was performed using the polymerase chain reaction method (PCR-RFLP), which is based on a certain portion of the multiple doubling of DNA in vitro with enzymes for obtaining DNA in the amount which is sufficient for visual perception.

In this case only the copied portion which satisfies these criteria and only if it is present in the sample was used for analysis. By means of PCR the introduction of mutations, splicing of DNA fragments is possible.

DNA was extracted from buccal epithelial using an ion exchange resin Chelex-100. Determination of CYP1A2 gene allele status at single nucleotide replacement (rs762551) was performed according to the common used methodology. Thermocycler amplifying "Tertsik" (DNA Technology, Russia) was used.

To amplify the CYP1A2 gene fragment that contained the polymorphic site (164A → C) oligonucleotide primers were used: forward is F: CCC AGA AGT GGA AAC TGA GA and reverse is R: GGG TTG AGA TGG AGA CAT TC.

Restriction of the amplification products was performed using endonuclease ApaI (MBI Thermo, Lithuania).

Restriction products were analyzed by electrophoresis in 2% agarose gel. As a molecular weight marker DNA pUC19 was used, hydrolyzed with MspI endonuclease (MBI Thermo, Lithuania).

The visualization of the amplification products and the restriction was carried out by staining the gel with ethidium bromide and photographing on transilyuminatore in ultra-violet light.

Restriction endonuclease fragment of 243 bp corresponded to A allele of CYP1A2 gene, and two restriction fragments of sizes 119 and 124 bp corresponded to C allele in an electrophoregram. The presence of all three bands indicates heterozygous genotype AC. According to the results of genotyping allele frequencies were calculated.

Results and discussion. Distribution of genotypes in the appropriate ratio of Hardy-Weinberg equilibrium was tested.

Comparing genotypes was performed using χ^2 test. Statistical hypothesis testing was conducted at a significance level of 0.05.

This study found the distribution of genotypes in the selected group: 35% AA, 50% AC, CC 15%, which is close to the European distribution. As for genotypes, only rs762551 (AA, homozygotes) was considered to be a genotype of rapid metabolizers. Individuals rs762551 (AC) – heterozygotes or rs762551 (CC) – homozygotes – were both considered to be slow metabolizers.

The frequency of alleles were as following: pA is 0.6 and qC is 0.4. According to the Hardy-Weinberg distribution, theoretically expected number of genotypes was calculated using the formula: p^2 AA: 2p (1-p) AC: $(1-p)^2$ CC.

Theoretically, the expected number of genotypes was as follows: 36% AA, 48% AC, 16% SS.

Testing the distribution of genotypes in the group for compliance with the Hardy-Weinberg showed that the population structure does not deviate from this equilibrium.

Conclusions:

1) as a result of study it was found the distribution of genotypes in the selected group (35% AA, 50% AC 15% CS).

2) the population structure does not deviate from the Hardy-Weinberg equilibrium.