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# *Effects of CYP2D6*4 Polymorphism in the Developmet of Ploygenetic Diseases in Chechen Poplulation*

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***Abstract*—The polymorphic variant of the CYP2D6 A18496G gene was studied as one of the most clinically significant in the MFD etiology in the Chechen population. Statistically significant effect of the GA genotype in relation to malignant breast tumors in women (*OR* = 1.93 at 95% *CI* = 1.20-3.09, *p* = 0.005) and the increase of the renal diseases risk and development of diabetes were revealed. Heterozygous genotype of patients with acute cerebrovascular disease ensures the protective effect.**

***Keywords—CYP2D6 polymorphism, diabetes mellitus, urogenital system, acute cerebrovascular disease, breast cancer, Chechen population***

## I. INTRODUCTION

Normal functioning of the body is formed at the molecular level and depends on the structure and functions of cell molecules involved in the metabolism, especially protein molecules. In turn, the structure and, accordingly, the functional capabilities of protein molecules are determined by genetic information within the nucleotide sequence of DNA. Information errors form a phenotypes variety, where single-nucleotide substitutions, forming the basis for gene polymorphism, play the leading role. Understanding the role of individual polymorphic gene variants in the development of complex phenotypes creates conditions for the genetic markers identification of predisposition to various polygenic diseases [1, 2].

The CYP2D6 gene, which determines the same-name enzyme of the xenobiotic activation phase, is mapped in the region of the chromosome 22 long arm and CYP2D6, and is highly polymorphic. Differences in enzymatic activity distributed human metabolizers into three phenotypic groups: “fast”, “slow” and “intermediate” metabolizers. The polymorphic variants CYP2D6*3 and CYP2D6*4 have the greatest clinical significance of the described CYP2D6 gene polymorphisms since they are responsible for the formation of human “slow” metabolizer phenotype without enzymatic activity. CYP2D6*4 polymorphism is the most extensive variant of the gene, the degree of incidence in European populations is 20-25%.

As evidenced by the number of publications in recent years, the participation of the enzyme in the metabolism of drugs determines the heightened interest in it by many scientists involved in pharmacogenomic study. CYP2D6 gene alleles demonstrate clinical efficacy of antidepressant medication, neuroleptic agents, antiarrhythmic drugs, antihypertensive agents, beta-blockers, and morphine derivatives [3].

It was found that the slowdown in the metabolism of antihypertensive drug debrisoquine is observed in homozygotes for CYP2D6*4 allele [4-7].

Some studies postulate the influence of CYP2D6*4 allele on tamoxifen metabolism in patients with hormone-sensitive breast cancer [8]. Monique J Bijl et al. report that the

antidepressants metabolism slows down in homozygous carriers of CYP2D6 *4 allele thus increasing the risk of side effects by 6 times [9].

On the basis of the mutant CYP2D6*4 allele functional inferiority, it is logical to assume its participation in the formation of complex phenotypes associated with increased risk of many diseases [10, 11], Parkinson's disease, systemic lupus erythematosus, pituitary adenoma, contrast nephropathy and ankylosing spondylitis [12, 13], coronary artery disease [14]. Bhat M.A. and Gandhi G (2018) studies note that recessive model in C2850T and the dominant model in G1846A are the best suitable models of inheritance to predict the gene effects (*OR*: 2.07, 95% *CI*: 1.05-4.08, $p = 0.031$ and *OR*: 1.70, 95% *CI*: 1.10-2.62, $p = 0.016$, respectively) [15].

These days, the contribution of CYP2D6 alleles to the malignant tumors formation is quite controversial. A number of studies demonstrated statistically significant associations of CYP2D6 polymorphism with the risk of cancer; other researchers have not found evidence of the involvement of the gene allelic variants in carcinogenesis [15]. Thus, the connection of CYP2D6*3 and CYP2D6*4 alleles with increased risk of acute lymphoblastic leukemia [16], squamous cell carcinoma of the head and neck was observed [17]. At the same time, the connection of the lung and bladder cancer development with CYP2D6 gene polymorphisms was not established [18, 17, 22].

Currently, polymorphism and features of multiple genes expression are studied; genetic tests are created on their basis, which improves the diagnostic accuracy, increases the potential for targeted therapy and positively contributes to the results of patients' treatment. However, in most cases the creation of unified genetic tests is problematic due to different effects of the same polymorphic genes variants in different populations, so the study in relatively isolated groups seems relevant. To solve this issue, we have studied the distribution of the most extensive CYP2D6 (MIM:12430) G1934A (allele CYP2D6*4) gene polymorphism of xenobiotics detoxification in the Chechen population and its role in the development of polygenic diseases.

## II. METHODS AND MATERIALS

We used the venous blood of the Chechen volunteers to study the polymorphic variant of the CYP2D6 gene. The ethnicity of respondents was determined by the questionnaire. Five groups were composed from the sample with total population of 679 people irrespective of their gender and age. The study design data are given in Table I.

The amplification products were separated by electrophoresis in agarose gel with addition of ethidium bromide as a dye. Amplicons were visualized in transmitted UV light by transilluminator.

Statistical analysis was performed by standard methods using the WinSTAT 2003.1 software package integrated in Excel. Equilibrium accordance was tested by the $\chi 2$ (Pearson) criterion; the population is considered equilibrium at $p>0.05$. The allele contribution in the risk of disease development was determined by the OR (odds ratio) with 95% confidence interval by the WinPepi program.

TABLE I. STUDY DESIGN OF THE *CYP2D6* (*CYP2D6**4) GENE POLYMORPHIC VARIANT

| No. | Sampling (clinically proven diagnosis) | Age | Biomaterial collection time | Collection place | Sample size |
|---|---|---|---|---|---|
| 1 | Acute cerebrovascular disease (ACVD) | 19-74 | 2013-2017 | Khanbiev U.I. Republican Clinical Hospital of Emergency Medical Care, Grozny | 154 |
| 2 | Breast cancer | 16-76 | 2010-2017 | Republican Oncology Center, Grozny | 123 |
| 3 | Diabetes mellitus type 2 | 37-69 | 2016-2017 | Republican Endocrinological Dispensary, Grozny | 86 |
| 4 | Kidney disease (glomerulonephritis, pyelonephritis) | 22-61 | 2018 | E.P. Glinka Republican Clinical Hospital, Grozny | 60 |
| 5 | Healthy individuals (control) | 21-78 | 2012-2018 | – | 256 |

The work was carried out at Chechen State University.

DNA was extracted from venous blood lymphocytes by Diatom ™ DNA Prep 200 kit (Isogen Ave., Moscow). Genotyping was implemented by the allele-specific tetraprimary PCR with subsequent electrophoretic detection in 2% agarose gel. The primers were used in PCR, the nucleotide sequence of primers was developed in the environmental genetics laboratory of the Institute of General Genetics (Table II). The method allows amplifying DNA fragments corresponding to alternative alleles in one test tube. Oligonucleotide primers (two external and two internal) were synthesized by Eurogen Ltd, Moscow. GenPak PCR Core reagent kits (Isogen laboratory, Moscow) were used to set the amplification reaction. The following conditions were selected for DNA fragment amplification: 94.0°C/5min. – 1 cycle; 94.0°C/30sec., 68.3°C/30sec., 72.0°C/30sec. – 32 cycles.

TABLE II. PRIMERS SEQUENCES FOR GENOTYPING

| 5'- 3' sequence | rs | Gene | Primer | Length |
|---|---|---|---|---|
| gtggatggtggggctaatgccttca t | 3892097 | ***CYP2D6*** | F | 463 |
| tctcgctccgcacctcgcgcagaa a | A1486G | | R | |
| catctcccacccccag | | | Fw | 190 |
| ggcgaaaggggcgtct | | | Rm | 313 |

## III. RESULTS

Deviations from the Hardy-Weinberg equilibrium were not revealed in the distribution analysis of polymorphism in the control sample and samples with acute cerebrovascular disorder (ACVD), diabetes mellitus (DM) and genitourinary system diseases (GSD). At the same time, the study of the genotypes distribution in the group with breast cancer (BC) for coherence with the equilibrium distribution of Hardy-Weinberg shows that for the studied CYP2D6*4 polymorphism there is a deviation from the equilibrium $\chi 2 = 7.735$; P=0.005 due to the prevalence of heterozygotes in the sample. The results of genotyping are given in Table III.

TABLE III. GENOTYPES FREQUENCY OF THE *CYP2D6* RS3892097 GENE POLYMORPHIC VARIANT

| **Groups** | | **ACVD** | | **BC** | | **DM** | | **GSD** | | **Control** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Individual N*** | | ***154*** | | ***123*** | | ***86*** | | ***60*** | | ***256*** | |
| Genotype frequency | GG | 79.22 | 122 | 65.85 | 81 | 72.09 | 62 | 66.67 | 40 | 78.81 | 212 |
| | GA | 20.78 | 32 | 34.15 | 42 | 27.91 | 24 | 33.33 | 20 | 21.18 | 57 |
| | AA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

It is important that homozygotes on the studied allele were not revealed in any of the studied groups, including the control group. Homozygous representatives were also not recorded in similar studies that were carried out in the Stavropol Krai and in Eastern Siberia [20].

High heterozygosity was observed in the group of patients with clinically established diagnosis of breast cancer and made 34.15%. The percentage of heterozygotes was equal to 33.33% in children with genitourinary system diseases. The frequency of CYP2D6*4 allele heterozygous carriers in the group of patients with ACVD reached 20.78%, in the group with diabetes it was 27.91%, in the control group the frequency of GA genotype amounted to 21.18%. The results obtained for the control group generally coincide with the values which were determined for the European populations by the allele CYP2D6*4.

According to subsequent associative analysis, the variability of heterozygotes frequency in the observed groups may indicate a relatively strong effect of polymorphism.

Associative analysis of heterozygous genotype frequency with the risk of multifactorial diseases (Table 4) revealed a significant effect of the GA genotype in relation of the breast cancer in women of the Chechen Republic: *OR* = 1.93 at 95% *CI* = 1.20-3.09, *p* = 0.005. Mutant CYP2D6*4 allele carrier also significantly increased the chance of kidney pathology: *OR* = 1.86 at 95% *CI* = 1.01-3.41 (*p* = 0.036). Heterozygous GA genotype also affects the risk of diabetes mellitus OR = 1.44, 95% C*I* = 0.83-2.50 (p>0.05). Interestingly, as the search of the link between the development of acute cerebrovascular disorder with presence of heterozygous genotype in individual shows, the latter has some protective effect, although the statistical significance of the difference does not reach: *OR* = 0.98 at 95% *CI* = 0.60-1.59 p>0.05.

1846 allele distribution frequency was also analyzed in the study of the corresponding samples. Allele frequency data are given in Table 5. The allele frequency was equal to 10.59% in the control, which generally corresponds to the indicators in European populations. This indicator reached its maximum in the group of Chechen women with BC and made 17.07%. In the patients' sample with ACVD, the lowest frequency of allele was observed compared with the other groups (10.38%).

TABLE IV. CYP2D6*4 ALLELE FREQUENCY IN THE STUDIED SAMPLES OF THE CHECHENS

| **Groups** | | **ACVD** | | **BC** | | **DM** | | **GSD** | | **Control** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Individual N*** | | ***154*** | | ***123*** | | ***86*** | | ***60*** | | ***256*** | |
| Genotype frequency | G | 89.61 | 276 | 82.93 | 246 | 86.04 | 172 | 83.33 | 120 | 89.41 | 538 |
| | A | 10.38 | 32 | 17.07 | 42 | 13.96 | 24 | 16.67 | 20 | 10.59 | 57 |
| | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

The comparative analysis of the frequency of the polymorphic variant CYP2D6 gene minor CYP2D6*4 allele in the BC group and the control group showed statistically significant differences for the A allele, *OR* = 1.61 at 95% *C*I= 1.05 to 2.47 (*p* = 0.031). The result suggests the effect of mutant allele on the process of normal cells transformation into malignant and on the development of breast cancer.

A similar effect of polymorphism is observed in relation to kidney disease in children of the Chechen population. The probability of developing pyelonephritis, glomerulonephritis increases in the A allele presence, which encodes a truncated protein: *OR* = 1.57 at 95% *CI* = 0.91-2.71. However, the differences do not reach any statistical significance (p>0.05).

The comparison of the allele frequency in the main group and the allele frequency in the group of patients with diabetes mellitus showed the increased risk of allele carriers, which influences the xenobiotics metabolism and is considered one of the multifactorial disease factors, *OR* = 1.32; 95% *CI* = 0.79-2.18. The influence of the studied polymorphism was observed at the trend level in patients with ACVD: *OR* = 1.09 at 95% *CI* = 0.69-1.73 (p>0.05).

## IV. CONCLUSIONS

The polymorphic variant of the CYP2D6 A18496G gene is one of the most clinically significant variants due to the loss of the enzyme functionality encoded by the gene. Homozygous allele carriers are characterized by a relatively weak metabolism of compounds that are substrates of CYP2D6 cytochrome. The activity of the enzyme in relation to 25% of drugs, some carcinogens, nicotine, antidepressants and other foreign compounds for the body was proved. Consequently, the interest of pharmacogenetics to this polymorphism has increased in recent years. In view of the CYP2D6 enzyme specifics, it is logical to assume that the A allele will be particularly important in relation to multifactorial diseases

(MFD) triggered by environmental factors, including xenobiotics. Furthermore, the degree of polymorphism involvement in MFD etiology will depend on the ethnicity of the study participants. Probably, the differences in the CYP2D6*4 variant distribution depend on both the ethnicity and the evolutionary prevailing habitat conditions. In this case, the differences can be explained by the action of natural selection in different geological residence zones. CYP2D6*4 allele frequency varies in different populations and reaches 10-16% among Europeoids, 3-12% among African populations. These data are indicated in Russian populations in full since polar nationalities live here: CYP2D6*4 occurs in Turkic representatives (Buryats, Bashkirs) at a frequency of 4.5-9.2%, in populations of Northern Siberia its frequency reaches 3.3-7.3% [19]. Within the given study the frequency of allele, causing the nonfunctional protein formation, reaches the value close to the Europeoid and makes 10.59%. Taking into account the consequences of single-nucleotide replacement of G on A in the splicing site, which lead to "slow metabolizer' phenotype formation, the degree of incidence frequency in the Chechen population and the level of environmental pollution should foster the pleiotropic effect in relation to the "action" of cytochrome on both endo- and exogenous compounds. On the one hand, allele carriership significantly increases the risk of multifactorial disorders, in particular breast cancer and genitourinary system diseases, on the other hand, it has a protective effect on the ACVD development. In our opinion, similar contradictory properties of polymorphic CYP2D6 gene variants can be explained by different degree of xenobiotics metabolism, which specifically affects the function of different systems and human organs. Hence, we believe that this study requires further consideration in order to detect xenobiotics with potential toxicity in the environment, where the enzyme encoded by the CYP2D6 gene takes part in biotransformation of xenobiotics.

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