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ASSOCIATION OF POLYMORPHIC G1934A VARIANT (ALLELE **4*) OF *CYP2D6* GENE WITH INCREASED RISK OF BREAST CANCER DEVELOPMENT IN UKRAINIAN WOMEN

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***Background*: Breast cancer (BC) is among the most common oncologic pathology in economically developed countries where it afflicts nearly 10% of women. Polymorphism of *CYP 2D6* gene is shown to be associated with increased risk of development of a number of pathologies, in particular cancer. *Aim*: The work was directed on evaluation of the role of polymorphism G1934A (allele **4*) of *CYP 2D6* gene in elevated risk of BC development in Ukrainian women. *Materials and Methods*: In the study there were enrolled 85 patients (group I) with histologically verified BC diagnosis of stages I and II. Clinical-genealogic study has been performed by the method of patient survey and following analysis of genealogy. Earlier obtained data on the frequency of genotypes and alleles of *CYP 2D6* gene in 637 Ukrainian people have been used as a control. For determination of allele variant **4* (*G1934A*) of *CYP 2D6* gene the method of PCR-RFLP has been used. *Results*: An increased risk of BC development in hereditary tainted patients with genotype **4*4* of *CYP 2D6* gene compared to the control group has been revealed. The frequency of **1*4* (IM) genotype has been found to be increased in the group of women with a family history of cancer (41.79%). Significant difference between the frequency of **1*1* (EM) and **1*4* (IM) genotypes in females with PR-positive and PR-negative tumors in the group of hereditary tainted patients has been registered. *Conclusion*: In conclusion, our study has revealed an increased risk of BC development in hereditary tainted patients compared to control group with genotype **4*4* (PM).**
***Key Words*: breast cancer, polymorphism, gene.**

Breast cancer (BC) is among the most common oncologic pathology in economically developed countries where it afflicts nearly 10% of women. The main causes of increased BC frequency account aging of population, prolonged influence of estrogens, and genome instability [1]. The CYP 2D6 mutant genotype has been reported to influence susceptibility to a number of cancers, such as lung, neck, liver cancer, melanoma and other types malignant diseases [14]. Numerous studies are devoted to the search of associations between the risk of BC development and individual patterns of the system of enzymes involved in metabolism of carcinogenes. Of doubtless interest are the studies directed on genetic polymorphism of enzymes that participate on metabolism of hormones, because the hereditary nature of hormonal signature of each individual and a prominent role of hyperestrogenemia in BC development have been demonstrated [2].

Among enzymes involved in metabolism of estrogens one could mention debrisoquine-4-hydroxylase (CYP 2D6) — an ensyme of phase I xenobiotic detoxification. It has been shown that CYP 2D6 takes part in the metabolism of 25% of all medicinal agents including cytotoxic preparations (tamoxyphen) used for BC therapy [5]. The enzymes activity of CYP 2D6 was only found in the liver, intestines and brain. Disease occurrence seems to be associated with the distribution of the cytochromes activity within different organs [15].

By the level of CYP 2D6 activity one could select poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM), and ultrarapid metabolizers (UM) [6].

CYP 2D6 enzyme demonstrates a wide spectrum of inter-individual variations in the rate of metabolism, and its activity levels differ in various population groups [7]. In the majority of cases, CYP 2D6 is capable to metabolise its substrates quickly. This level of activity of the enzyme (EM) is associated with genotype that is responding to wild type homozygote — genotype **1*1*.

CYP 2D6 gene is localized on chromosome 22q13.1. (OMIM *124030). In white European population in *CYP 2D6* gene the most often found substitution is G1934A (allele *4) on the line between intron 3 and exon 4, the presence of which leads to incorrect mRNA splicing resulting in a shift of reading frame, termination of translation, and generation of defective protein product lacking enzymatic activity (Fig. 1) [8].

G to A substitution is identified as a primary defect in *CYP 2D6* locus, and by various estimations it is present in 80–90% of mutant alleles of poor metabolizers [9]. It has been shown that allele **4* is associated with decreased *CYP 2D6* activity [8]. In nearly 5–10% of white European population there have been detected mutations in both alleles of *CYP 2D6* gene — genotype **4*4* related to PM.

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Abbreviations used: BC – breast cancer; CI – confidence interval; CYP 2D6 – cytochrome P450 2D6; ER – estrogen-receptor; EM – extensive metabolizers; IM – intermediate metabolizers; mRNA – messenger ribonucleic acid; OMIM – online mendelian inheritance in man; OR – Odds Ratio; PCR-RFLP – polymerase chain reaction-restriction fragment length polymorphism; PM – poor metabolizers; PR – progesterone receptor; UM – ultrarapid metabolizer.

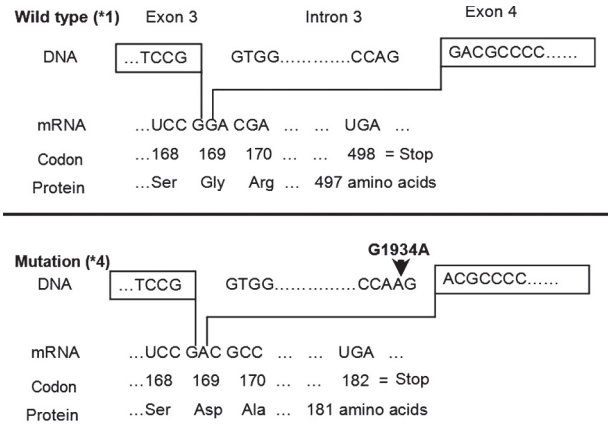

Fig. 1. G1934A polymorphism in intron 3 of *CYP2D6* gene

The carriers of only one allele **4* (heterozygous state) are intermediate metabolizers (IM) of CYP 2D6 substrates — genotype **1*4*.

CYP2D6 participates in metabolic activation of procarcinogens, so, in patients with EM phenotype compared to other groups with lower enzyme activity, larger amounts of reaction-active compounds may be formed what in turn may lead to malignant transformation. However, the results of comparative evaluation of the role of *CYP 2D6* allele variants in cancer development are conflicting [10, 11]. Apart from this, polymorphism of *CYP 2D6* gene is thought to be related to the increased risk of the development of a number of diseases, including some types of cancer [3, 4].

In Ukraine, only general frequency of alleles and genotypes by polymorphic variant G1934A (allele *4) of *CYP 2D6* gene has been studied [12], but the relation between the frequency of genotypes of this polymorphic variant with increased risk of BC development has not been studied yet.

The aim of our study was to evaluate the role of genetic polymorphism G1934A (allele *4) of *CYP 2D6* gene in elevated risk of breast cancer development in Ukrainian women.

MATERIALS AND METHODS

The study protocol was approved by the Committee for the Ethical Issues of P.L. Shupik National Medical Academy of Post-Graduate Education. All patients have signed an informed consent.

In the study there were enrolled 85 female ethnic Ukrainian patients (group I) from 18 to 80 years old (average age — 45.25±13.26 years) with histologically verified diagnosis of lobular and/or ductal BC of stages I and II, who received treatment in Kyiv Municipal Oncological clinics. All patients were involved in clinico-genealogic study by the method of survey where all information has been fixed in a specially developed file with the following analysis of genealogy. According to the results of such clinico-genealogic study all patients were randomized in two subgroups: group Ia — 67 patients who have 2 or more BC cases among the relatives of I–II degree of kin (hereditary tainted patients); group Ib — 18 patients who have no relatives with cancer (hereditary untainted patients). The control group was represented with Ukrainian people (n=637, average age 50.73±19.3 years). During the study of population frequency of CYP 2D6*4 genotypes in females and males and individuals from different age groups we have registered no significant differences, that's why we have united them into one group (control group).

Genomic DNA was extracted from the peripheral leukocytes using standard procedures. For determination of allele variant **4* (*G1934A*) of *CYP 2D6* gene the method of PCR-RFLP (polymerase chain reaction — restriction fragment length polymorphism) has been used. PCR-RFLP was carried out by the method of Brown et al. [13]. Amplification reaction was performed in thermocycler Applied Biosystems 2700. DNA was denatured for 3 min at +94 °C, with the following 30 cycles at +94 °C for 40 s, at +60 °C for 40 s, and at +72 °C for 40 s.

The products of amplification (421 b.p. in length) underwent restriction with restriction endonuclease BstNI (MvaI) (Fermentas, Lietuva), and restriction products were electrophoretically separated in 2% agarose gel and visualized with the use of ViTran system.

Homozygotes by *CYP 2D6*4* (A1934A — **4*4*) were identified by the presence of two fragments with the length of 77 and 344 b.p., and homozygotes by wild type (G1934G — **1*1*) — by the presence of three fragments with the length of 77, 161 and 183 b.p. Heterozygotes (G1934A — **1*4*) were presented by four fragments with the length of 77, 161, 183 and 344 b.p. (Fig. 2).

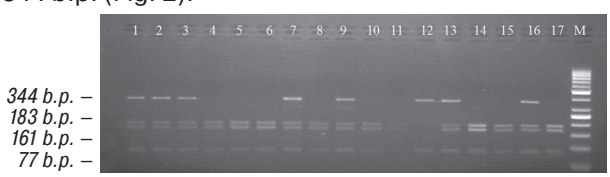

Fig. 2. Electrophoretic analysis of restriction fragments of *CYP 2D6* gene in 2% agarose gel. *CYP 2D6 *1*1*– lines 4, 5, 6, 8, 10, 11, 14, 15; 17; *CYP2D6 *1*4* — lines 1, 2, 3, 7, 9, 13, 16; *CYP 2D6 *4*4* — line 12; M — molecular weight marker

The data were analyzed with the use of Statistica 6.0 program. For determination of significance of differences between genotype frequencies in compared groups, standard x-square criterion (χ^2) was used. Odds Ratio (OR) has been used for evaluation of relative risk of disease development for each genotype. In all types of analysis, values $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

When we have compared group I of BC patients with the control group, significant difference between the frequencies of genotypes and alleles for **1*1* (EM) and **4*4* (PM) of CYP 2D6 gene has been registered (Table 1).

In a separate analysis of distribution of genotypes and alleles by *CYP 2D6* in hereditary tainted BC patients and control group (Tables 1 and 2), there has been registered higher increase of the risk of BC de-

velopment in females from subgroup Ia with genotype **4*4*, not only in comparison with control group but also in comparison with the whole group of BC patients (OR 3.59 and 2.77 respectively).

Table 1. Distribution of *CYP 2D6* genotypes and alleles in groups I and II

Gene polymorphism	Genotypes, alleles	Group I n = 85		Control group n = 637		χ^2	OR	CI
		n	%	n	%			
*CYP2D6*4*	EM (*1*1)	45	52.94	417	65.46	5.1	0.59	0.38–0.94
	IM (*1*4)	34	40.00	203	31.87	2.25	1.43	0.9–2.27
	PM (*4*4)	6	7.06	17	2.67	4.69	2.77	1.06–7.23
	p (*1)	0.73		0.81		6.81	0.62	0.43–0.89
	q (*4)	0.27		0.19			1.62	1.12–2.34

Table 2. Polymorphism of *CYP 2D6* gene in hereditary tainted patients

Gene polymorphism	Genotypes, alleles	Group Ia n = 67		Control group n = 637		χ^2	OR	CI
		n	%	n	%			
*CYP2D6*4*	EM (*1*1)	33	49.25	417	65.46	6.91	0.51	0.31–0.85
	IM (*1*4)	28	41.79	203	31.87	2.71	1.53	0.92–2.56
	PM (*4*4)	6	8.96	17	2.67	7.58	3.59	1.36–9.44
	p (*1)	0.7		0.81		9.71	0.54	0.36–0.8
	q (*4)	0.3		0.19			1.86	1.25–2.77

This fact evidences on increased risk of BC development in hereditary tainted patients compared to control group. The frequency of genotype **1*4* (IM) was found to be increased in the group of females with family history of cancer (41.79%). The presence of genotype **1*1* (EM) may have a protective effect what is evidenced by OR value.

The comparison of the data obtained in hereditary untainted patients group (group Ib) and other groups in the study did not reveal significant differences in the frequencies of alleles and genotypes of *CYP 2D6* gene what could be explained by a small number of patients in this group.

During the study of genotyping by *CYP 2D6* gene we have revealed (Table 3) that distribution of frequencies of alleles and genotypes of *CYP 2D6* gene significantly differs from Mendel's distribution. We did not find explanation for the meaning of non-Mendel's distribution of the frequences of *CYP 2D6* gene and genotypes in the literature. By our opinion, the causes of such distribution could be: 1) age of mutation: if the mutation appeared relatively recently, it has no time to be accumulated, so the dominant majority of individuals still have just one allele of defect gene (heterozygous status — **1*4*), as it occurs in general population (control group); 2) influence on reproductive function — sex hormones (progesterone and estrogen) belong to endogenous substrates of CYP 2D6 in human body, that's why the decrease of enzyme activity disturbs their metabolism what affects the development of pregnancy; 3) early prenatal death of fetus with genotype **4*4* may lead to decreased accumulation of this genotype among population in total; 4) postnatal selectivity — increase of adaptive capabilities in individuals with certain genotype. Estimation of real cause of such distribution should be studied separately.

Table 3. Distribution of *CYP 2D6* genotypes and alleles in the studied groups

		*1*1 (EM)		*1*4 (IM)		*4*4 (PM)		*1*4 + *4*4		Allele frequency	
		n	%	n	%	n	%	n	%	p (*1)	q (*4)
Group I n=85		45	52.94	34	40	6	7.06	40	47	0.73	0.27
Group I	Group Ia n=67	33	49.25	28	41.79	6	8.96	34	51	0.7	0.3
	Group Ib n=18	12	66.67	6	33.33	0	0	6	50	0.83	0.17
Control group n=637		417	65.46	203	31.87	17	2.67	220	34	0.81	0.19

Our results have shown significant elevation of the frequency of heterozygotes **1*4* (IM) in BC patients and as a consequence, an increase of the number of individuals carrying at least one allele **4* (**1*4*+**4*4*). As far as homozygosity by allele **4* forms phenotype PM [8], we have united homozygotes (PM) and heterozygotes (IM) by allele **4* and have registered significant increase of the frequency of allele **4* (47.06%) carriage compared to control group (34.54%) (OR (CI 95%): 1.68 (1.07–2.66); χ^2=5.1).

The analysis of receptor status of BC patients did not reveal significant difference between the frequencies of genotypes by *CYP 2D6* for estrogen receptor-positive (ER+) and estrogen-receptor-negative (ER–) tumors. However, there has been registered significant increase of the frequency of heterozygotes **1*4* and decrease of homozygotes **1*1* in patients with progesterone receptor positive (PR+) and progesterone receptor-negative (PR–) tumors in the group of hereditary tainted BC patients (Table 4).

Table 4. *CYP2D6* polymorphism and receptor status

Gene polymorphism	Genotypes, alleles	Group Ia PR^+ n = 37		Group Ia PR^- n = 30		p	χ^2	OR	CI
		n	%	n	%				
*CYP2D6*4*	EM (*1*1)	14	37.89	19	63.33	0.05	4.31	0.35	0.13-0.95
	IM (*1*4)	20	54.05	8	26.67	0.02	5.11	3.24	1.15-9.11
	PM (*4*4)	3	8.11	3	10	1	0.07	0.79	0.15-4.25
	p (*1)	0.65		0.77		0.18	2.2	0.56	0.26-1.21
	q (*4)	0.35		0.23				1.78	0.83-3.83

In conclusion, our study has revealed an increased risk of BC development in hereditary tainted patients compared to control group with genotype **4*4* (PM) of *CYP 2D6* gene. The frequency of genotype **1*4* (IM) has been found to be elevated in the group of women with family history of cancer (41.79%). The highest frequency of heterozygotes was found in hereditary tainted BC patients, while in BC patients without family history of cancer it was found to be close to that in control group. Alsowe have revealed a significant difference in the frequency of genotypes **1*1* (EM) and **1*4* (IM) in hereditary tainted BC patients with PR^+ and PR^-tumors.

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