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Possibility of clinical application of polymorphic variants of the genes ESR1 and CYP2D6*4 in patients with breast and endometrial cancer

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The objective: **determining gene polymorphism features ERS1, CYP2D6 in patients with breast cancer (RHZ) and endometrial cancer (EC) and the impact assessment studied genetic characteristics compared to receptor sta; tus (immunohistochemical determination of expression levels of ER, PR) tumors and the results of the treatment.**

***Patients and methods*. Article presents the results of complex clinical, morphological, clinical-genealogical, and molecular-genetic examination of 28 females: 19 patients with breast cancer (BC), 9 patients with endometrial cancer (EC), including 5 patients with primary-multiple tumors (PMT) with and without tumor pathology aggregation in families.**

***Results*. The It was determined that in patients' families malignant tumors of breast, uterine body and/or ovaries prevail that corresponds to Lynch type II syndrome (family cancer syndrome). Molecular-genetic examination of genomic DNA of peripheral blood and histological sections for the presence of SNPs of *ESR* and *CYP2D6*4* genes comparing with the results of immunohistochemical study of tumors for receptors ER and PR status have not found associations between these characteristics; although among EC patients the occurrence of genotypes *397TT* and *351AA* was significantly higher comparing with BC patients (55,55% and 10,5% for genotype *397TT*,and 15.8% for genotype *351AA*, respectively). At the same time the patients with BC and primary-multiple tumors (PMT) of female reproductive system organs (FRSO) that carried mutations in *BRCA1* in all the cases demonstrated positive ER and PR receptor status and adverse combinations of polymorphous variants of the genes *ESR1 (397CC, 397TC)* and *CYP2D6*4 (1846G, 1846GA)*, suggesting combined effect of these factors on the development of malignant neoplasias of FRSO in families with positive family cancer history. In BC patients, receiving standard hormone therapy with tamoxifen, those, who had genotype *1846GG* of the gene *CYP2D6*4*, in 3 patients (15,8%) of 19 (100%) patients disease recurrence was diagnosed.**

***Conclusion*. The obtained results allow clinical use of the assessment of polymorphism frequency of the genes *ESR1* and *CYP2D6*4* for selection of individual hormone therapy regimens schemes for BC patients, to increase efficacy of dispensary observation after finishing of special therapy for such patients, and also personalization of complex and combined treatment regimens.**

Key words*: breast cancer, endometrial cancer, family cancer syndrome, single nucleotide polymorphisms (SNPs) of the genes ESR1, CYP2D6*4.*

Modern stage of oncology development is marked by improvement of traditional ways of prevention and diagnostics for malignant tumors of various geneses. Increased incidence of cancer of breast, ovaries, uterine body and other organs brings to the forefront novel methods of cancer early diagnostics and prevention that can be based on molecular biology achievements. Therefore, medical-genetic counseling and genetic testing of patients with malignant processes get increasingly growing widespread.

The first stage during medical-genetic counseling is an examination of cancer family history by clinical genealogical investigation of proband's (the person, who's family tree is observed) family history and determination of number of patients with cancer of different localizations in several generations of a family. The second stage – is testing of proband and family members for occurrence of mutations of genes-suppressors of tumor growth, namely *BRCA1* and *BRCA2*, and also for polymorphism of estrogen receptor gene *ERS1*. Determination of such genetic alterations is an evidence of genetic component existence in tumor disease [1–4].

According to recent research data, the family of cytochrome oxidases CYP has important role in the processes of neutralization of the majority of exogenous detrimental compounds, endogenous metabolites, and drugs. Activity of CYP enzymes that implement the first step of detoxification depends on structure of their coding genes. In separate cases at high activity of the enzymes of first detoxification step and low activity of enzymes of second detoxification step the increase of harmful effect on organism is observed due to the growth of cellular toxicity of intermediate compounds and their insufficient clearance from organism. Apart of this, because of indicated properties, metabolic processes of drugs transformation have different targeting and they influence on treatment efficiency and the risk of toxic effects development.

It is recognized that the enzyme CYP2D6 on cellular level is involved in transformation of almost 90% of detrimental compounds, endogeneous metabolites and drugs; and features and rate of metabolic transformations determine both the risk of oncologic diseases development and peculiarities of response to treatment or susceptibility to cytostatic therapy. Formation of patients' phenotype of increased susceptibility to detrimental factors action and adverse effects in drugs administration is associated with inherited alteration in gene structure. Allele variant of the gene *CYP2D6*4* is presented by single nucleotide variation *G1846A* in gene sequence; in its occurrence the enzyme with reduced or missing catalytic activity is produced. In one Ukrainian study it was demonstrated that indicated polymorphous variant of the gene modified the risk of the development of benign pathology and cancer of female reproductive system organs (FRSO) [5].

Most of published works to date have examined the mechanisms of reduction of tamoxifen sensitivity in the presence of certain allele variants of the gene *CYP2D6*. The enzyme *CYP2D6* was found necessary for tamoxifen transformation into intermediate active metabolite endoxifen, possessing therapeutic anti-estrogen activity, and genetic variants influence on rate and efficacy of active metabolite generation and response to treatment.

During preliminary analysis of family cases of malignant and benign pathology in females of Cherkassy region we determined the

growth of risk for tumors development depending on presence in patients of polymorphous variants of the gene *ESR1* that have an effect on reduction of receptors sensitivity and hyperestrogenemia development [4, 5]. The assessment of combined effect of estrogen receptors status and environmental pro-cancerogens, activating in the first detoxification step, on neoplasias risk and their course and also on treatment results have not been studied yet. Therefore, according to different authors' data, complex analysis, considering tumors histological properties, is required [6–12].

The objective: of this work was determination of peculiarities of polymorphism of the genes *ERS1, CYP2D6* in patients with cancer of breast and uterine body, and also assessment of the effect of examined genetic peculiarities, comparing with receptor status (immunohistochemical determination of expression level for ER, PR) of tumors and results of received treatment.

PATIENTS AND METHODS

Over the last five years we are providing clinical, clinical-genealogical and molecular-genetic studies in patients with malignant tumors of female reproductive system organs (FRSO). For this purpose we use questionnaire method to obtain clinical-genealogical data; primary patients with BC and UBC filled the questionnaire, developed by us, and then they received complete medical-genetic counseling by oncogynecologist. After clinical-genealogical analysis of family trees the presence or absence of family cancer syndrome FCS) was determined. Molecular-genetic methods included identification of polymorphous variants *T-397C, A-351G* of the gene *ERS1* and of the gene *CYP2D6*4(G1846A)* in peripheral blood and surgical material by multiplex polymerase chain reaction (PCR). Molecular-genetic study was provided in SE «Reference centre for molecular diagnostics of the Ministry of Public Health of Ukraine».

The study involved 28/100% patients with FRSO cancer: 19/67.9% with malignant tumors of breast and 9/32,1% patients with endometrial cancer, including 5 females in whom synchronous or metachronous primary-multiple tumors (PMT) of FRSO were diagnosed: two patients with bilateral breast cancer (BC) and metachronous ovarian cancer (OC) in 2 and 3 years, respectively; one patient with BC and OC which developed also metachronously in 2 years; one patient with BC with metachronous OC in 7 years; one patient with BC, which developed metachronously in 3 years after treatment for thyroid cancer. All examined females had Ukrainian nationality.

All probands received complete physical examination according to examination standards, adopted in Ukraine. During interview with proband and during filling the questionnaire we paid attention to the following data: number of relatives of I–III degree of kinship, suffering with cancer, their family relation to proband. Clinical-genealogical analysis was provided according to Amsterdam criteria II (three or more relatives with Lynch-associated tumors – colorectal cancer, cancer of breast, uterus, ovaries, stomach and others, at that one of oncologic patients should have I degree of kinship with other relatives, and malignant cancers – at least in two generations). In the majority of female patients from the study group both in BC (68,4%), and in EC (55,5%) family cancer syndrome (FCS) of Lynch II type was determined after family tree analysis (Tabl. 1). All patients with BC and EC that underwent clinical-genealogical examination received medical care, including surgical, complex or combined treatment for cancer according to treatment standards in Ukraine in the CE «Cherkassy Regional Oncologic Dispensary» of Cherkassy Regional Council. Tumor process stage in patients with cancer was assessed according to FIGO classification, tumors verification was performed by morphologist. In surgical materials of tumors of all patients with BC and EC immunohistochemical studies were performed for detection of ER and PR expression levels. All the probands gave their written consent for use of results of the study of their biological material for scientific research results.

Molecular-genetic study of polymorphous variants of the genes *ESR (A-351G, T-397C)* and *CYP2D6*4 (G1846A)* was performed in peripheral blood samples of 28 patients with BC (n=19) and EC (n=9). Peripheral blood collection was provided into 2,5 ml sterile tubes of closed system «Monovette» with ethylenediamine tetraacetate (EDTA) manufactured by the company «Sarstedt». Sterile tubes with collected material were stored at -20 єС (not more than 1 month) in refrigerating chambers before transportation to State Establishment «Reference Centre for Molecular Diagnostics of the MPH of Ukraine» (Kyiv). Samples transportation was performed in frozen state in cold containers. For DNA isolation from peripheral blood commercial kit "DNA-sorb-B" was applied (according to manufacturer's instruction). Determination of studied genes polymorphism was provided using polymerase chain reaction (PCR) with reagents of the company Thermo Scientific, for PCR performance modified protocol with appropriate primers was applied. The state of obtained amplification fragment was analyzed with electrophoresis in 2% agarose gel. Amplicons were subjected to hydrolytic degradation by restriction endonucleases, obtained frag-

Table 1

Distribution and characteristics of FRSO malignant tumors in examined female patients (n=28)

Malignant pathology	Number of female patients examined n=28 (100%)	Family cancer syndrome (FCS) presence according to family trees analysis	Without tumor pathology aggregation in family trees
Breast cancer, n=19 (100%)	19 (67,8)	13 (68,4)	6 (31,6)
Endometrial cancer, n=9 (100%)	9 (32,2)	5 (55,5)	4 (45,5)
Primary-multiple tumors (combination of FRSO malignant tumors), n=5 (100%)	+ 5 (17,9)	-	5 (100)

Table 2

Distribution of probands (patients with BC and EC) by age and age median

Female patients examined	Age (years)					
	29-30	31-40	41-50	51-60	61-70	71-72
BC patients, n=19 (%)	1 (5,3)	4 (21,1)	11 (57,8)	3 (15,8)	-	-
Median Average age, years	44 44,6					
EC patients n=9 (%)	-	1 (11,1)	1 (11,1)	2 (22,2)	5 (55,5)	-
Median Average age, years	56 57,2					
Totally, n=28 (100%) of patients	1 (3,5)	5 (17,9)	12 (42,8)	5 (17,9)	5 (17,9)	-

Table 3

Reproductive-menstrual status of examined patients with breast cancer and endometrial cancer

Characteristics		Patients with FRSO cancer, n=28 (100%)	
Onset of menarche	Before age of 12	28 (100)	9 (32,1)
	12-15 years		17 (60,7)
	Older than 15 years		2 (7,1)
Number of deliveries	0	28 (100) (21 patient had deliveries)	7 (25)
	1-2 deliveries		17 (60,7)
	More than 3		4 (14,3)
Number of abortions	0	28 (100)	20 (71,4)
	1-2		1 (3,6)
	More than 3		7 (25)
Number of miscarriages	0	28	26 (92,9)
	1-2		2 (7,1)
	More than 3		-
Lactation	Was absent	28 (100)	
	up to 6 months		-
	6-12 months		21 (75)
	>1 year		-
Number of menstrual days	Up to 3 days	19 (100)	-
	4-6 days		17 (89,5)
	7 days and more		2 (10,5)
Menstrual duration	Regular (24-32 days)	28 (100)	28 (100)
	Irregular (more than 2 days)		-
Menopause duration	Up to 5 years	9 (100)	3 (33,3)
	5-10 years		1 (11,1)
	more than 10 years		5 (55,6)
FRSO surgeries in anamnesis	Were absent	-	-
	In uterine appendages		-
	In breasts		-
Tumor diagnostics	At prophylactic examination	9 (UBC after US examination 19 (BC) -	9 (32,2)
	Self-reported		19 (67,8)
	By oncologist		-

ments were analyzed with the method of restriction fragment length polymorphism (RFLP). Restriction was carried out in microthermostat at a temperature 37 °C for 12 hours.

Restriction of polymorphous variants of *ESR1* gene was terminated by heating at a temperature of 65 °C for 20 minutes. The obtained fragments were analyzed with electrophoresis in 2% agarose gel with addition of ethidium bromide and further visualization by computer system Vitran.

For determination of polymorphous variants of the gene *CYP2D6*4 (G1846A)* modified protocols with oligonucleotide primers using PCR (polymerase chain reaction) and further analysis of restriction fragment length polymorphism (RFLP) was applied. Specific fragments of the gene *CYP2D6*4 (G1846A)* were amplified using commercial kit DreamTaqGreen PCR MasterMix (of the company «ThermoScientific», USA) in accordance to the conditions of the reaction.

The tubes with prepared amplification mixture were placed into thermocycler «FlexCyclerBU» (Analytic Jena, Germany) to ensure proper temperature regimen of polymerase chain reaction.

Restriction was provided in microthermostate at 37 °C for 12 hours. Amplified fragments state was analyzed in 3% agarose gel (agasose of the company «ThermoScientific», USA), with addition of ethidium bromide, molecular weight marker GeneRuler 50 bpDNALadder («ThermoScientific», USA), following with visualization by computer system Vitran. The obtained results were visualized in transilluminator.

Statistical processing was carried out with programs MS Excel 2010 and Statistica 10. To analyze the differences of obtained numerical indices variation statistics methods were used, and for genotypes frequency – Pearson's criterion χ^2 was applied, for less then ten samples Yate's correction was applied and odds ratio (OR) was calculated with confidence interval (95% confidence interval, CI).

RESULTS AND THEIR DISCUSSION

Among the total number of examined females (n=28/100%) the largest was the number of patients with BC (n=19/67,8%), EC (n=9/32,2%); among these patients in 5 cases metachronous or synchronous PMT were found (n=5/17,9%), these data are presented in the Tabl. 1. The age of the patients with FRSO from families with positive cancer history and without tumor pathology aggregation in their family trees (Tabl. 1) ranged in similar margins – from 29 to 72 years (Tabl. 2). The values of average age

Table 4

Malignant tumors incidence in probands' relatives, suffering from cancer, according to family trees data

Data of oncogenetic counseling and clinical-genealogical analysis of family trees		Patients with FRSO cancer (n=28/100%), of them in 11/39,3% FCSs were determined (47/100% relatives, suffering from cancer in family trees of 28 probands)
I degree of kinship (those who had 2 and more relatives)	Maternal lineage	Suffered from cancer 11 (23,4%)
	Paternal lineage	Suffered from cancer 8 (17,0%)
II degree of kinship (2 and more relatives, up to 6)	Maternal lineage	Suffered from cancer 19 (40,4%)
	Paternal lineage	Suffered from cancer 9 (19,2%)

and median for decade had significant differences in the groups of examined females with BC and EC: breast cancer developed in patients, on average, 10 years earlier than uterine body cancer (p?0,05).

Reproductive-menstrual status of examined females with FRSO cancer is presented in the Tabl. 3; according to obstetric anamnesis data no statistically significant differences were determined between groups of patients with BC and EC, therefore in the table they are presented together.

As the table demonstrates, in patients with BC and EC menarche more frequently was observed in the age 12–15 years, also the majority of females (more than 70%) did not have abortions and miscarriages in anamnesis, and the number of deliveries were in a range 1–2 per lifespan. 75% of females had deliveries, apart from this, lactation duration was in a range 6–12 months, and menstrual cycle in all female patients examined was regular; all of them did not have surgeries in appendages and breasts in the past. The majority of BC patients detected tumors by themselves; further these tumors were verified during oncologist's examination. Talking about EC, all of them were detected at gynecologist's prophylactic examination followed with evaluation in small pelvis US examination with vaginal probe that further led to diagnostic curettage and endometrial cancer verification. The above dictates the need and importance of health communication among population concerning timely cancer detection to increase awareness of females, having relatives with cancer in their families. The above dictates the need for clinical-genealogical analysis of family trees at primary examination of patients by family doctor, gynecologist or other specialists.

The analysis of clinical-genealogical data in 28 family trees of patients with BC (n=19) and UBC (n=9) has revealed malignant pathology aggregation, in particular, 47 (100%) cancer cases in families, among them FRSO malignant tumors dominated: EC – 8 (17%), BC – 9 (19,1%), OC – 2 (4,3%) and also there were 14 (29,8%) cases of cancer of gastro-intestinal tract (GIT) organs – colorectal cancer, stomach cancer, pancreatic cancer and other tumors localizations (cervical cancer, lung cancer, laryngeal cancer and others). Incidence of FRSO cancer, hormone-dependent tumors was rather high and comprised 33 (70,2%).

Resulting from clinical-genealogical analysis of family trees of 28 (100%) patients with BC and EC (aged from 29 to 72 years) in 18 (64,3%) cases where there were 2–6 relatives of I–II degree on kinship, suffering from cancer (Lynch II syndrome), probands' relatives on the maternal lineage more often suffered from cancer (30/63,8%), than the relatives on paternal lineage (17/36,2%) that is addressed in the Tabl. 4. During family tree analysis (Tabl. 1) statistically reliable increase of the incidence of family cancer syndrome in BC patients comparing with EC patients (68,4% and 55,5%, respectively) was discoverd.

All primary patients with BC and EC who received medical-genetic counseling, regardless of the presence or absence of malignant tumors aggregation in their family trees, were provided with molecular-genetic study of histological material of excised tumors or peripheral blood for detection of mutations in the genes *BRCA1 (185delAG, 5382insC)* and *BRCA2 (6174delT),* which results were described in details in previous publication [3], polymorphism of

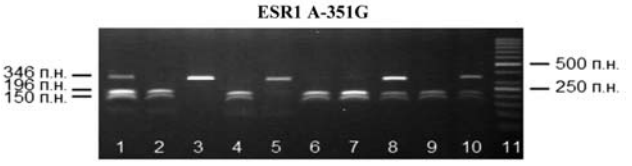

Fig. 1. Electrophoregram of restriction analysis of products of ESR1 (A-351G) gene fragment.
Samples: 1, 8, 10 - genotype AG; 2, 4, 6, 7, 9 - genotype AA; 3, 5 - genotype GG; 11 - molecular weight marker

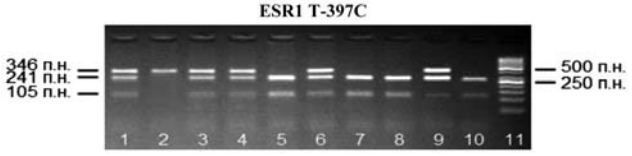

Fig. 2. Electrophoregram of restriction analysis of products of ESR1 (T-397C) gene fragment.
Samples: 1, 3, 4, 6, 9 - genotype TC; 2 - genotype CC; 5, 7, 8, 10 - genotype TT; 11 - molecular weight marker

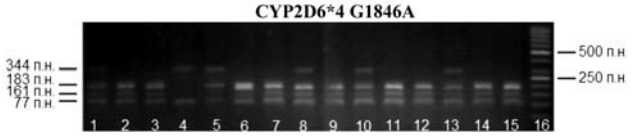

Fig. 3. Electrophoregram of restriction analysis of polymorphism of CYP2D6*4G1846A.
Samples: 1, 5, 8, 10, 13 - genotype GA, samples 2, 3, 6, 7, 9, 11, 12, 14, 15 - genotype GG, sample 4 - genotype AA, 16 - molecular weight marker

the genes *ESR1 (A-351G, T-397C)* and *CYP2D6*4(G1846A)*.

Molecular-biological study of polymorphisms of the gene *ESR1 (A-351G, T-397C)* comprised the detection of restriction analysis products of fragment of the gene *ESR1* in BC and EC tumors, which results are presented in the Figs. 1 and 2.

According to the results of histological and immunohistochemical examination of the material of excised tumors it was found that in BC G2-3 differentiation grade (moderately and low-differentiated tumors) was observed more frequently, in particular, in 15 (78,9%) of 19 (100%) patients with breast cancer, and in EC – G1 (high-differentiated tumors) in 6 (75%) of 8 (100%) patients with uterine body cancer. Provided molecular-genetic testing for mutations in the genes *BRCA1,2* in examined patients has not revealed in any case mutations in the gene *BRCA2*, but in 5 patients, notably with BC and with FCS according to family trees data, the same mutation was determined – *5382insC* in the gene *BRCA1* (Tabl. 5).

Molecular-genetic study of genomic DNA of peripheral blood and histological tumor sections for SNP of the genes – *ESR1, CYP2D6*4*y in general group of examined patients with BC and EC did not revel associations with immunohistochemical status of detected tumors. The genotypes for which at previous analysis we

Table 5

Comparative analysis of differentiation grade data and receptor status of FRSO malignant tumors and the results of molecular-genetic study in patients with BC and EC

Case number	Diagnosis	Grade G	ESR1 T397C	ESR1 A351G	BRCA1	BRCA2	Cyp2D6 G1846A	ER	PR
107-III	BC+OC	3	CC	GG	**+5382insC**	-	GG	2+	2+
101	BC	2	TC	AG	-	-	GA	2+	2+
91- III	bilateral BC+OC	2+2+2	TT	AA	-	-	GG	2+	1+
83	BC	2	TC	AG	-	-	GG	2+	2+
76	BC	2	TC	AG	**+5382insC**	-	GA	1+	1+
70	BC	3	CC	AG	-	-	GA	1+	1+
68	BC	1	CC	AG	-	-	GG	1+	1+
64	BC	2	TC	AG	-	-	GA	2+	3+
63	BC	3	TC	AG	**+5382insC**	-	GA	1+	1+
60 - III	BC+thyroid cancer	2	CC	AG	-	-	GG	1+	1+
59	BC	3	TC	AG	-	-	GG	1+	1+
54	BC bilateral+OC	2+2+2	TT	AA	**+5382insC**	-	GA	2+	2+
53	BC	2	TC	AG	-	-	GG	2+	2+
42	BC	1	CC	GG	-	-	GA	-	-
15	BC	1	TC	AG	-	-	GA	1+	1+
27	BC	3	TC	AG	-	-	GG	-	-
24	BC	3	TC	AG	-	-	GG	-	-
23	BC	3	TC	AA	**+5382insC**	-	GA	1+	-
37	BC	1	TC	AG	-	-	GG	1+	1+
*PMT (n=5)									
106	EC	3	TT	AA	-	-	GG	2	2+
96	EC	3	TT	AA	-	-	GG	1+	2
35	EC	1	TT	AA	-	-	GG	2+	2+
33	*BC+OC	2+1	TC	AG	-	-	GG	1+	2+
28	EC	2	TT	AA	-	-	GG	2+	2+
20	EC	1	TC	AG	-	-	GG	2+	2+
16	EC	1	TT	AA	-	-	GG	1+	3+
13	EC	1	CC	GG	-	-	GA	2+	2+
7	EC	1	CC	GG	-	-	GG	2+	2+

*BC - 19, of them 5 PMT (2 with bilateral BC+OC, 1 with BC+OC, 1 with BC+EC, 1 patient with BC and thyroid cancer),*EC - 9 (1 of them is included in study of both groups of patients, as this patien was diagnosed with PMT - EC+BC.

determined the relation with malignancies development, were similarly distributed in patients with positive levels of ER and PR expression. However, all EC patients had positive receptor status of estrogen and progesterone receptors – 9 of 9/100%, and in BC – in 16/84,2% patients of 19/100% positive status of two receptors was found (10/52,6% cases of BC with molecular type luminal type A and 6/31,6% – with luminal type B), at the same time in 3/15,8% patients triple negative molecular type of BC was diagnosed (based on the data of expression levels of membrane protein of epidermal growth factor receptor her2/neu, which is associated with the negative prognosis of disease course). Individually, her2/neu-positive molecular type of BC in examined group was not detected.

Comparing distribution of genotypes of the gene *ERS1* in patients with BC and EC we determined that among patients with uterine body cancer genotypes *397TT* and *351AA* were more frequent than in patients with breast cancer (55,55% and 10,5% with genotype *397TT* and 15,8% with genotype *351AA*, respectively) what is shown in the Tabl. 6.

At the same time in patients with BC and primary-multiple tumors (PMT) of female reproductive system organs (FRSO) who were the carriers of the mutation in the gene *BRCA1* in all cases positive receptor status in ER and PR, and also adverse combinations of polymorphous variants of the genes *ESR1 (397CC, 397TC)* and *CYP2D6*4 (1846GA)* was determined that suggests combined effect of above factors on the development of FRSO malignancies in families with cancer positive family history.

In BC patients, receiving standard hormone therapy with tamoxifen (20 mg daily for 5 years after finishing of complex or combined BC treatment) we determined combinations of genotypes *1846GA* of the gene *CYP2D6*4* and *ESR1* (397 TC, 397CC; 351 AG, 351GG) in 3 (75%) of 4 (100%) of patients with disease recurrences (in 2-3 years after special treatment). The patient with combination of genotypes *CYP2D6*4* (1846GA)*/ESR1(*397CC,351GG) died of recurrence. The obtained results of clinical application of the assessment of polymorphism frequency of the genes *ESR1, CYP2D6*4* can be used for selection of individual regimen of chemotherapy treatment (considering *CYP2D6* role in drug metabolism, in particular, cytostatics) and hormone therapy (taking into account the data about the absence of tamoxifen transformation into active substance in human organism in the presence of adverse polymorphisms of the

Table 6

Distribution of polymorphous variants of the genes ESR1 (A-351G, T-397C) and CYP2D6*4 (G1846A) in patients with BC and EC

Patients with FRSO cancer, n=28	ESR1 (T-397C)	ESR1 (A-351G)	CYP2D6*4 (G1846A)
BC (n=19/100%)	TT – 2/10,5% TC – 12/63,2% CC – 5/26,3%	AA – 3/15,8% AG – 14/73,7% GG – 2/10,5%	GG – 10/52,6% GA – 9/47,4% AA – 0
EC (n=9/100%)	TT – 5/55,5% TC – 2/22,2% CC – 2/22,2%	AA – 5/55,5% AG – 2/22,2% GG – 2/22,2%	GG – 8/88,9% GA – 1/11,1% AA – 0

gene *CYP2D6*4*) in BC patients to increase treatment efficiency due to treatment personalization and improvement of patients' total survival prognosis.

Therefore, under the same conditions and genetic predisposition, determined with several factors, female patients with the genotype *397TT* will be more frequently diagnosed with EC. As shown in the Tabl. 5, 6, we discovered in histological sections from four patients the mutation of the gene *BRCA1* in patients with BC and PMT with positive receptor status. In all these four patients the adverse polymorphous variants of the genes *ERS1* and *CYP2D6* were found; and three patients had their combinations. The peculiarities were have discovered suggest combined effect of above mentioned factors on malignancies development in families with malignant tumors aggregation and indicate the need for complex molecular-genetic examination of such patients and their relatives.

Our results open the perspective of a novel strategy of further studies of the problem of early diagnostics and prevention for FRSO malignancies. It focuses not only on detection of families with aggregation of malignant tumors in their family trees, but also in determination of the carriers of mutations of genes-suppressors *BRCA1* and *BRCA2,* polymorphous variants of the genes *ESR1* and *Cyp2D6*4* in families with FCS or in PMTs detection. Establishment of clinical-genetic register of such persons within certain regions of Ukraine to address issues concerning monitoring of their health, prevention and pre-clinical diagnostics of hereditary forms of FRSO cancer and other tumors within the frame of family cancer syndrome is one of the actual tasks of clinical oncology.

CONCLUSIONS

1. Clinical-genealogical family trees analysis of 28 (100%) patients with BC and EC (aged from 29 to 72 years) determined tumor association in 18 (64,3%) families of patients with BC and EC that corresponded to family cancer syndrome (Lynch 2 syndrome). At that, relatives on the maternal lineage more often suffered from cancer (30/63,8%), than relatives on the paternal lineage (17/36,2%).

2. Molecular-genetic examination of genomic DNA of peripheral blood and histological sections for the presence of SNPs of *ESR* and *CYP2D6*4* genes, comparing with the results of immunohistochemical study of tumors for receptors ER and PR status, did not find associations between these characteristics; although among EC patients the occurrence of genotypes *397TT* and *351AA* was significantly higher comparing with BC patients (55,55% and 10,5% for genotype *397TT*, and 15,8% for genotype *351AA*, respectively).

3. Also in patients with BC and PMT of FRSO that were the carriers of the mutation in the gene *BRCA1* in all the cases positive receptor status in ER and PR and adverse combinations of polymorphous variants of the genes *ESR1 (397CC, 397TC)* and *CYP2D6*4 (1846GA)* were determined that suggests combined effect of above factors on the development of FRSO malignancies in families with cancer positive family history.

4. In BC patients that have recurrence after application of standard hormone therapy with tamoxifen we found combinations of genotypes *1846GA* of the gene *CYP2D6*4* and *ESR1* (397 TC, 397CC; 351 AG, 351GG) in 75 % of cases.

5. Our results suggest the necessity of clinical-genealogical and molecular-biological examinations in patients with BC and EC both with positive and with negative cancer family history to increase efficacy of special treatment due to its personalization. And determination of SNPs of the gene *CYP2D6*4,* considering its role in metabolizing not only cancerogens, but also drugs, including tamoxifen, will further assist planning of individual regimens of hormone therapy and chemotherapy in patients with FRSO cancer.

Можливості клінічного використання тестування на поліморфні варіанти генів ESR1 та CYP2D6*4 у хворих на рак грудної залози та ендометрія

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Мета дослідження: визначення особливостей поліморфізму генів *ERS1, CYP2D6* у хворих на рак грудної залози (РГЗ) і рак ендометрія (РЕ) та оцінювання впливу вивчених генетичних особливостей у порівнянні з рецепторним статусом (імуногістохімічне визначення рівнів експресії ER, PR) пухлин і результатами проведеного лікування.

Матеріали та методи. Було здійснено комплексне клінічне, морфологічне, клініко-генеалогічне і молекулярно-генетичне обстеження 28 жінок: 19 – хворих на РГЗ та 9 – хворих на РЕ, у тому числі 5 хворих із первинно-множинними пухлинами (ПМП) з та без агрегації пухлинної патології у родинах.

Результати. Установлено, що у родинах хворих спостерігаються злоякісні пухлини переважно грудної залози, тіла матки та/або яєчників і травного тракту, що відповідає синдрому Лінча II типу (сімейний раковий синдром). Молекулярно-генетичне дослідження геномної ДНК периферійної крові та гістологічних зрізів пухлин на SNP генів *ESR1, CYP2D6*4* у порівнянні з результатами імуногістохімічного дослідження пухлин на рецепторний статус ER, PR у загальній групі обстежених не виявило асоціацій між ними, але у пацієнток з РЕ вірогідно частіше фіксували генотипи *397TT* і *351AA* порівняно із хворими на РГЗ (55,55% та 10,5% за генотипом *397TT* і 15,8% за генотипом *351AA* відповідно). У той самий час у пацієнток із РГЗ та первинно-множинними пухлинами (ПМП) органів жіночої репродуктивної системи (ОЖРС), які були носіями мутацій у гені *BRCA1,* був виявлений в усіх випадках позитивний рецептурний статус за ER та PR і несприятливі комбінації поліморфних варіантів генів *ESR1 (397CC, 397TC)* та *CYP2D6*4 (1846GG, 1846GA)*. Це свідчить про комбінований вплив зазначених чинників на розвиток злоякісних новоутворень ОЖРС у родинах із обтяженим на рак сімейним анамнезом. У хворих на РГЗ, які отримували стандартну гормонотерапію тамоксифеном, за наявності генотипу *1846GG* гена *CYP2D6*4* у 3 (15,8%) з 19 (100%) хворих був діагностований рецидив захворювання.

Заключення. Одержані результати дозволяють клінічно використовувати оцінювання частоти поліморфізмів генів *ESR1, CYP2D6*4* для підбору індивідуальної схеми гормонотерапії у хворих на рак грудної залози та підвищення ефективності диспансерного спостереження після закінчення спеціальної терапії таких пацієнток і персоналізації схем комплексного і комбінованого лікування.

Ключові слова*: рак грудної залози, рак ендометрія, родоводи, сімейний раковий синдром, однонуклеотидні заміни, поліморфізми (SNP) генів ESR1,CYP2D6*4.*

Возможности клинического использования тестирования на полиморфные варианты генов ESR1 и CYP2D6*4 у больных раком грудной железы и эндометрия

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Цель исследования: определение особенностей полиморфизма генов ERS1, CYP2D6 у больных раком грудной железы (РГЖ) и раком эндометрия (РЭ) и оценка влияния изученных генетических особенностей по сравнению с рецепторным статусом (иммуногистохимическое определение уровней экспрессии ER, PR) опухолей и результатами проведенного лечения.

Материалы и методы. Было выполнено комплексное клиническое, морфологическое, клинико-генеалогическое и молекулярно-генетическое обследование 28 женщин: 19 – больных РГЖ, 9 – больных РЭ, в том числе 5 больных с первично-множественными опухолями (ПМО), с и без агрегации опухолевой патологии в семьях.

Результаты. Установлено, что в семьях больных наблюдаются злокачественные опухоли преимущественно грудной железы, тела матки и/или яичников и пищеварительного тракта, что соответствует синдрому Линча II типа (семейный раковый синдром). Молекулярно-генетическое исследование геномной ДНК периферической крови и гистологических срезов опухолей на SNP генов *ESR1, CYP2D6*4* в сравнении с результатами иммуногистохимического исследования опухоли на рецепторный статус ER, PR в общей группе обследованных не выявило ассоциаций между ними, но у пациенток с РЭ вероятно чаще фиксировали генотипы *397TT* и *351AA* по сравнению с больными РГЖ (55,55% и 10,5% по генотипу *397TT* и 15,8% по генотипу *351AA* соответственно). В то же время у пациенток с РГЖ и ПМО органов женской репродуктивной системы (ОЖРС), которые были носителями мутаций в гене *BRCA1,* был выявлен во всех случаях позитивный рецепторный статус по ER и PR и неблагоприятные комбинации полиморфных вариантов генов *ESR1 (397CC, 397TC)* и *CYP2D6*4 (1846GG, 1846GA).* Это свидетельствует о комбинированном влиянии отмеченных причин на развитие злокачественных новообразований ОЖРС в семьях с осложненным раком семейным анамнезом. У больных с РГЖ, которые получали стандартную гормонотерапию тамоксифеном, при наличии генотипа *1846GG* гена *CYP2D6*4* у 3 (15,8%) из 19 (100%) больных был диагностирован рецидив заболевания.

Заключение. Полученные результаты позволяют клинически использовать оценку частоты полиморфизмов генов *ESR1, CYP2D6*4* для подбора индивидуальной схемы гормонотерапии у больных с раком грудной железы и повышения эффективности диспансерного наблюдения после окончания специальной терапии таких пациенток и персонализации схем комплексного и комбинированного лечения.

Ключевые слова*: рак грудной железы, рак эндометрия, родословные, семейный раковый синдром, однонуклеотидные замены, полиморфизмы (SNP) генов ESR1, CYP2D6*4.*

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