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***Key Words**: cancer of female reproductive system organs, family trees, family cancer syndrome, single nucleotide substitutions, polymorphisms (SNP) of the gene ESR1.*

CLINICAL SIGNIFICANCE OF ESTROGEN RECEPTOR GENE *ERS1* SNP IN CANCER PATIENTS FROM FAMILIES WITH ONCOLOGICAL PATHOLOGY IN PEDIGREES

***Aim**: to determine genetic risk of oncologic pathology development in family trees of probands from families with family cancer history and patients with primary-multiple tumors basing on the results of clinical-genealogical examination and genetic analysis of estrogen receptor gene ESR1 in patients with ovarian cancer and breast cancer. **Methods:** clinical, morphological, clinical-genealogical and molecular-genetic studies. **Results:** it was presented the results of complex of 145 females: 90 patients with cancer of female reproductive system organs (FRSO), including patients with primary-multiple tumors with aggregation of tumor pathology in families, and 55 females — control group without cancer family history. It was determined that in families of patients with FRSO cancers the malignant tumors of FRSO, gastro-intestinal tract and others prevail that corresponds to Lynch II syndrome (family cancer syndrome). Molecular-genetic examination of single nucleotide polymorphism (SNP) of the gene ESR1 in genomic DNA of peripheral blood and from histological material determined significant increase the frequency of 397CC genotype of the gene ESR1 in cancer patients and aggregation of malignant pathology in families comparing with frequency of this genotype in female of control group. Statistical processing of these results demonstrated that the risk of malignant pathology development (odds ratio — OR, 95% confidence interval — CI) increases in 4.95 times in case of determination of CC genotype of the gene ESR1 (T-397C), and in case of heterozygous carriage of this gene — in 2.25 times. The presence of TT genotype of the gene ESR1 (T-397C) reduces the risk of the disease (OR = 0.21). **Conclusion:** the frequency of AA, AG, GG genotypes of the gene ESR1 (A-351G) also differed in cancer patients comparing with these characteristics in healthy females: significant reduction AA genotype AA to 33.33% versus 67.27% in healthy females (p = 0.001), significant increase of AG genotype from 32.73 to 57.78% (p = 0.006), tendency of genotype GG increase — from zero to 8.89% (p = 0.057). This is a basis for clinical-genealogical and molecular genetic study in females with FRSO malignant pathology for detection of polymorphisms (SNP) of estrogen receptor gene (ESR1). The examined SNPs polymorphisms of ESR1 can be predictors of cancer development and aggregation of malignant tumors in proband's family and progeny.*

The mechanisms of cells malignant transformation and predisposition to breast cancer (BC), ovarian cancer (OC) and other organs cancer represent the most actual oncologic problem. Increased attention to patients with BC and OC is based on epidemiological, clinical and biological peculiarities of these tumors. BC belongs to the most common forms of malignant tumors worldwide without tendency towards decrease of morbidity, and has multiple clinical-biological and variable clinical course [1]. OC refers to the tumors with mysterious pathogenesis, with high morbidity and mortality of patients resulting from late diagnosis in spite of possibilities of ultrasound diagnostics of the pathology in early stages [2]. These cancer forms are often observed in several generations of patients, and sometimes — also as primary multiple tumors (PMT) in single person [3]. The problem of PMT, both synchronous and metachronous, also refers to important issues that require detailed study due to greater life expectancy of population, including patients after treatment of female reproductive system organs (FRSO) cancer and colon cancer (CC). In PMT pathogenesis genetic predisposition to cancer incidence in also important due to genome instability which also may be associated with specific patients' treatment, and also with influence of radiogenic, chemical factors and others on human body in modern conditions of life.

Achievements of genetic and molecular biology extended knowledge of the nature of cells malignization and role of genes in tumor growth [4–8]. Mutation in

the genes — suppressors of tumor grown *BRCA1* and *BRCA2* refer to genetic alterations which may facilitate tumor development [9–12]. Most clearly the role of gene mutations in predisposition to cancer development may be followed on the example of family cancer syndrome (Lynch syndrome) [13, 14].

Currently, in the mechanisms of predisposition to cancer development, including its hereditary variants, the attention is paid not only to mutations in the genes *BRCA1* and *BRCA2,* but also to the mutations in the genes of DNA reparation: *CHEK2, ATM, BRIP1 (FANCJ), PALB2 (FANCN), RAD51C (FANCO)*, among which the gene *CHEK2* was recognized as a gene of multi-organic predisposition to cancer development [15]. Clinical and molecular-genetic studies in various countries determined the role of single nucleotide polymorphism (SNP) also as a marker of predisposition to BC and other forms of pathology [16–19], although the mechanisms of potential malignization of cells with SNP involvement need further investigations. In this context, attention is drawn to SNP of estrogen receptor gene *ERS1* that is involved in hormonal metabolism of normal cells and hormonal pathogenesis of a number of malignant tumors [17], in particular, BC and OC. Mutations of estrogen receptor gene *ERS1* lead to disorder of estrogen signaling and inter-gene interactions, and to alteration of hormonal status of cells and tissues. Therefore, in the recent years there was a growing number of studies dedicated to the role of *SNP* of *ERS1* gene. Considering the importance of hormonal homeostasis disorders in development and progression neoplasms, study of *SNP* of *ERS1* is important for determination of genetic risk for cancer development in FRSO.

Clinical studies demonstrated that increased risk of cancer development in members of families with tumor pathology aggregation really exists, but the issue of what exact factors or its complex facilitate neoplasias development in proband's relatives remains unspecified. Therefore, the priority direction in modern oncology is a search for molecular markers which application would contribute to the definition of persons with the highest risk of malignization. Knowledge of such markers would form a basis for its application in the development of clinical protocols of examination of members of families with family cancer syndrome.

***Aim of the work*:** to determine genetic risk of oncologic pathology development in pedigrees of probands from families with family cancer history and patients with PMT basing on the results of clinical-genealogical examination and genetic analysis of estrogen receptor gene *ESR1* in patients with OC and BC.

OBJECT AND METHODS

The study enrolled 90 patients with FRSO cancer (BC, OC, primary multiple FRSO tumors) from families with cancer family history and 55 practically healthy females without cancer cases in their pedigrees. All examined females were Ukrainians and lived in Cherkassy region of Ukraine.

All probands (cancer patients and practically healthy females) independently completed their clinical-genealogical maps that further were analyzed and complemented by oncologist-gynecologist during personal interview with proband and also at reviewing of medical documentation after probands complex examination according to examination standards of patients adopted in Ukraine. During interview with proband the attention was paid to the following data: number of relatives, suffering from cancer, of I–III degree of kindship, their family relationship to proband. Clinical-genealogical analysis was implemented according to Amsterdam criteria II (three of more relatives with Lynch-associated tumors — colorectal cancer, BC, OC, gastric cancer (GC) and others, at that one of oncologic patients should have I degree of kindship with other relatives, and malignant tumors — at least in two generations). In examined healthy females there were no relatives with cancer in three generations of family tree. All cancer patients that underwent clinical-genealogical examination received medical assistance, including surgical, complex or combined treatment according to the Ukrainian standards of patients' treatment in CE «Cherkassy Regional Oncologic Dispensary» (Cherkassy, Ukraine). The stage of tumor process in cancer patients was evaluated according to FIGO classification, tumors verification was carried out by morphologist. All probands gave written consent to application of their biological material in scientific research.

Genomic DNA for molecular-genetic study was isolated from histological sections of paraffin blocks of surgical material in 46 patients. The material was transported to the laboratory of SE «Reference Centre for Molecular Diagnostics of the Ministry of Public Health (MPH) of Ukraine». For deparaffinization 750 μl of 100% xylol were added to paraffin tumor sections, they were treated in vortexer for 15 s and incubated at room temperature for 1 hour in thermoshaker. The samples were centrifuged for 1 min at 12,000 g, supernatant was removed. Deparaffinization procedure was repeated for one time more. To remove the xylol remains after deparaffinization the samples were washed in four steps. At the first step 1 ml of 100% ethanol was added to the material, after that the samples were treated in vortexer for 10 s, defecated for 5 min at room temperature in thermoshaker and supernatant was removed. The first step was repeated for one time more. Second and third steps corresponded to the first step, beside the fact that 1 ml of 95% ethanol and 1 ml of 75% ethanol, respectively, were added to the samples. At final step the samples were washed in 1 ml of ddiH_2O, treated in vortexer for 10 s, incubated for 5 min in thermoshaker and centrifuged for 1 min at 12,000, after that supernatant was removed.

DNA isolation from histological material was carried out with commercial test-system *«Quick-DNA™ Universal Kit»* (Zymo Research, USA) according to instructions for the kit. Into 1.5 μl centrifuge tube with the material which was purified from paraffin and xylol 45 μl of ddiH_2O, 45 μl of *Solid Tissue Buffer* and 10 μl of *Proteinase K* were added. The material was incuba-

ted for 12 hours at 55 °C, periodically treated in vortexer for 10–15 s. After incubation the tubes were kept at 94 °C for 20 min. Then 600 μl of *Genomic Binding Buffer* was added to the samples and the material was treated with vortexer for 10–15 s, after that the samples were centrifuged at 12,000 g for 1 min. Supernatant was transferred to the column *Zymo-Spin*™ *IIC-XL* that was situated in collector tube and centrifuged for 1 min at 12,000 g. Then 400 μl of *DNA Pre-Wash Buffer* was added to the column with new collector tube, it was centrifuged for 1 min at 12,000 g and the liquid was removed from the collector tube. Then 700 μl of *g-DNA Wash Buffer* was applied to the column and the sample was centrifuged 1 min at 12,000 g, the liquid after centrifugation was removed from the collector tube. One more time 200 μl of *g-DNA Wash Buffer* was applied to the column and the sample was centrifuged at 14,000 g for 1 min. The column was transferred to clean microtube and 60 μl of *DNA Elution Buffer* was applied directly to column filter, after that the sample was incubated for 5 min at room temperature. The columns, inserted to centrifuge mictotubes, were centrifuged for 1 min at 14,000 g for DNA elution. The supernatant, containing purified DNA, was used for polymerase chain reaction (PCR).

Molecular-genetic study of polymorphous variants of *ESR* gene *(A-351G, T-397C)* was carried out in peripheral blood samples of 44 patient with BC (n = 21) and OC (n = 23). Also molecular-genetic study of peripheral blood for the same polymorphisms occurrence was provided in 55 practically healthy females from control group that, according to family trees data, did not have hereditary risk factors of malignant pathology development.

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 °C (not more than 1 month) in refrigerating chamber 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 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 fragments 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 65 °C for 20 min. The obtained fragments were analyzed with electrophoresis in 2% agarose gel with addition of ethidium bromide and further visualization by computer system Vitran.

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 (CI; 95% CI).

RESULTS AND DISCUSSION

Among the total number of examined females (n = 90) the number of patients with OC (n = 23/25.6%) and BC (n = 21/23.3%) was almost the same, and the number of patients with PMT pathology was 46/51.1% (Table 1). Age of patients with FRSO cancer from families with cancer family history was in the same range — from 21 to 75 years (Table 2). The values of average age and median for decade did not have significant differences in the groups of examined females ($p > 0.05$).

Table 1

Distribution and characteristics of FRSO tumors in examined female patients (n = 90) with cancer family history

Pathology	Number of female patients examined, n (%) (n = 90)
OC	23 (25.6)
BC	21 (23.3)
PMT (combination of malignant tumors of FRSO and gastro-intestinal tract)	46 (51.1)

Table 2

Distribution of probands (patients with FRSO cancer from families with cancer family history and healthy females without cancer cases in their family trees — control group) by average age and age median

Probands	Average age and median, years					
	21–30	31–40	41–50	51–60	51–70	>70
Patients with FRSO cancer from families with cancer positive family history, n = 90						
Average age	28.6	36.5	46.5	54.7	64	73
Age median	29	33	41	53	65	72
Healthy females without cancer cases in their family trees, n = 55 (control group)						
Average age	27.7	35.6	46.6	56.0	–	–
Age median	29	39	48	52	–	–

Reproductive-menstrual status of examined patients with FRSO cancer and females of control group is presented in the Table 3.

As can be seen from the Table 3, in patients, comparing with healthy females, significantly more frequently ($p < 0.05$) menarche was observed in earlier age, and also they had greater number of deliveries and miscarriages. In 20% of cancer patients lactation was absent, there were two times more patients with menstrual duration 3 days, at that in 23.3% of patients their menstrual cycle was irregular, 37.8% of patients had in their anamnesis surgeries for benign processes in breast and uterine appendages, whereas healthy females did not have any surgical interventions in FRSO in anamnesis. The tumors in patients were diagnosed at prophylactic examinations (26.7%), by oncologist (44.4%), while in 28.0% of females FRSO tumors were

Table 3

Reproductive-menstrual status of examined patients with FRSO cancer and females of control group

Characteristics		Patients with FRSO cancer, n (%) (n = 90)		Healthy females (control group), n (%) (n = 55)	
Onset of menarche	Before age of 12	90	20 (22.2)	55	3 (5.5)
	12–15 years		57 (63.3)		45 (81.8)
	Older than 15 years		13 (14.5)		7 (12.7)
Number of deliveries	0	90	3 (3.3)	55	11 (20.0)
	1–2 deliveries		59 (65.5)		40 (72.7)
	More than 3		28 (31.1)		4 (7.3)
Number of abortions	0	90	54 (60.0)	55	35 (63.6)
	1–2		30 (33.3)		15 (27.3)
	More than 3		6 (6.7)		5 (9.1)
Number of miscarriages	0	90	74 (82.2)	55	0
	1–2		10 (11.1)		0
	More than 3		6 (6.7)		0
Lactation	Was absent	18	18 (20.0)	0	0
	Lactation:				
	till 6 months	72	30 (41.7)	44	8 (18.2)
	6–12 months		26 (36.1)		14 (31.8)
	> 1 year		16 (22.2)		22 (50.0)
Number of menstrual days	Up to 3 days	90	19 (21.1)	55	6 (10.9)
	4–6 days		58 (64.4)		42 (76.4)
	7 days and more		13 (14.5)		7 (12.7)
Menstrual duration	Regular (24–32 days)	90	69 (76.7)	55	53 (96.3)
	Irregular (more than 32 days)		21 (23.3)		2 (3.6)
Menopause duration	Up to 5 years	36	10 (27.8)	15	5 (33.3)
	5–10 years		10 (27.8)		5 (33.3)
	More than 10 years		16 (44.4)		5 (33.3)
FRSO surgeries in anamnesis	Were absent	90	56 (62.2)	55	0
	In uterine appendages		9 (10.0)		0
	In breasts		25 (27.8)		0
Tumor diagnostics	At prophylactic examination	90	24 (26.7)	55	0
	Self-reported		26 (28.9)		0
	By oncologist		40 (44.4)		0

self-reported that indicated insufficient outreach to the public regarding early diagnosis of cancer and relatives ignorance as to the existence of cancer patients in their families. The above dictates the need for clinical-genealogical analysis of family pedigrees at primary examination of patients by oncogynecologist or another specialists.

The analysis of clinical-genealogical data in pedigrees of cancer patients has revealed a wide range of tumor pathology — 321 tumors of different genesis, among them CC prevailed (31.5%). BC incidence was 23.0%, uterine body cancer — 21.2%, OC — 18.1%, in other words, incidence of tumors of hormone-dependent organs was rather large and comprised 62.3%. GC demonstrated the lowest rate among all the tumors (6.2%) (Table 4).

Table 4

Tumors distribution in family trees of probands (considering proband's tumors)

Malignant tumors	Number of tumors, n (%)
Endometrial cancer (EC)	68 (21.2)
OC	58 (18.1)
BC	74 (23.0)
CC	101 (31.5)
GC	20 (6.2)
Total	**321 (100)**

Therefore, resulting from clinical-genealogical examination of patients, tumors association in family trees in the form of family cancer syndrome was determined that was also demonstrated by probands' pedigrees (Fig. 1–4).

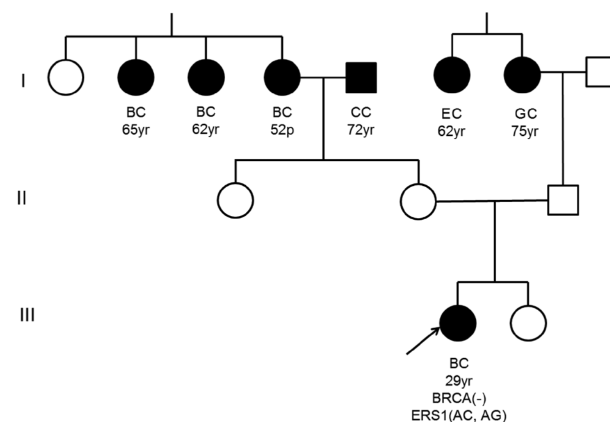

Fig. 1. Pedigree of ***proband Ch.I.V.***, 29 years (observation No. 53). Family cancer syndrome. Proband has BC stage I. In I generation of pedigree — tree patients with BC in the age of 52; 62; 65 years, CC (72 years), EC — (62 years), GC (65 years). The results of proband's molecular-genetic examination: SNP of the gene *ERS1 (AC, AG,)*, genes *BRCA 1/2* (−) Legend: yr — years

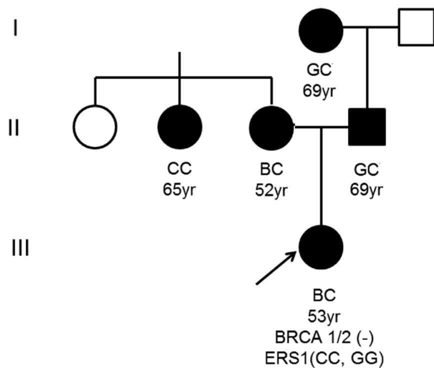

Fig. 2. Pedigree of ***proband G.S.N.***, 53 years (observation No. 58). Family cancer syndrome. Proband has BC stage I. In her mother — BC (52 years), in her father — GC (69 years), and patient with CC (65 years), and patient (69 years) — GC. The results of proband's molecular-genetic examination: SNP of the gene *ERS1 (CC, GG),* genes *BRCA 1/2* (–)
Legend: yr — years

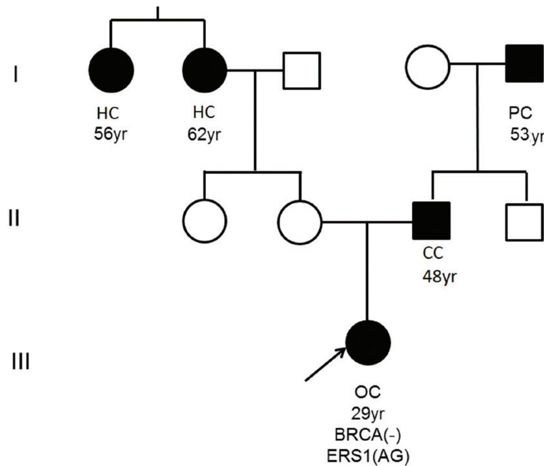

Fig. 3. Pedigree of ***proband T.I.V.***, 29 years (observation No. 78). Family cancer syndrome. Proband has OC. In her pedigree — aggregation of malignant tumors: two patients with hepar cancer (56 and 62 years), prostate cancer (53 years), CC (48 years). The results of proband's molecular-genetic examination: SNP of the gene *ERS1 (AG),* gene *BRCA 1* (–)
Legend: PC — prostate cancer; HC — hepar cancer; yr — years

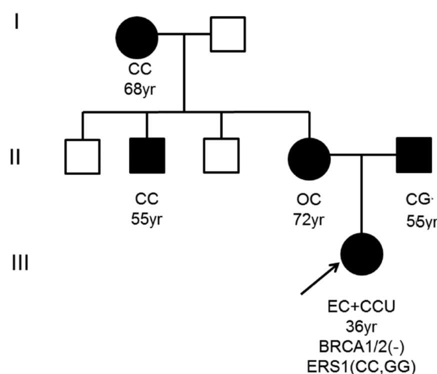

Fig. 4. Pedigree of ***proband S.G.V.***, 36 years (observation No. 13). Proband has primary-multiple cancer — EC (adenocarcinoma) and cancer colli uteri (squamous cell carcinoma). Family cancer syndrome. In her mother — OC (72 years), in father — GC (55 years). In I and II generations — CC in 68 and 55 years. The results of proband's molecular-genetic examination: SNP of the gene *ERS1* (*CC, GG*), gene *BRCA 1/2* (–)
Legend: CCU — cancer colli uteri; yr — years

Molecular-genetic examination comprised the detection of products of restriction analysis of gene *ESR1* fragment in BC and OC tumors which results are presented in the Fig. 5 and 6.

ESR1 A-351G

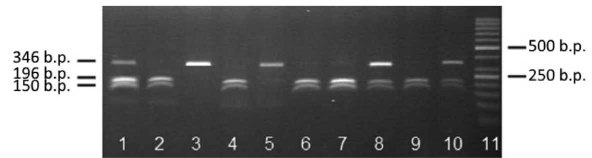

Fig. 5. Electrophoregram of restriction analysis products of *ESR1* gene fragment (A-351G). Samples: 1, 8, 10 — genotype AG*;* 2, 4, 6, 7, 9 — genotype AA*;* 3, 5 — genotype GG; 11 — molecular weight marker

ESR1 T-397C

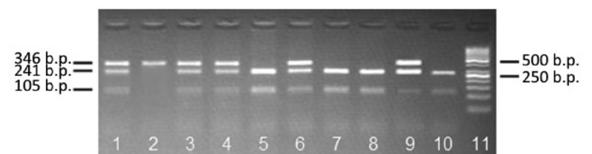

Fig. 6. Electrophoregram of restriction analysis products of *ESR1* gene fragment (T-397C). Samples: 1, 3, 4, 6, 9 — genotype TC*;* 2 — genotype CC; 5, 7, 8, 10 — genotype TT; 11 — molecular weight marker

Basing on the results of molecular-genetic tumors examination different genotypes frequency of the gene *ESR1* in examined cancer patients comparing with examination results of healthy females was determined (Table 5). According to the results of statistical processing of the results in cancer patients the frequency of genotype TT of the gene *ESR1* (T-397C) was lower comparing with control data and comprised 25.56% *versus* 61.82% (p = 0.001). In contrast, the frequency of genotypes TC and CC of the same polymorphous variant of the gene *ESR1* (T-397C) was significantly higher and comprised 52.22% *versus* 32.73% (p = 0,034) and 22.22% *versus* 5.45%, respectively (p = 0.014).

The frequency of genotypes AA, AG, GG of the gene *ESR1* (A-351G) also was different in cancer patients comparing with these indices in healthy females: significant reduction of genotype AA frequency up to 33.33% *versus* 67.27% in healthy females (p = 0.001), significant increase of genotype AG frequency from 32.73 to 57.78% (p = 0.006), tendency to increase of genotype GG frequency — from zero to 8.89% (p = 0.057).

The largest relative risk of cancer development (OR = 4.95) at 95% CI was determined for genotype CC of polymorphous variant T-397C of the gene *ESR1*, two times lower relative risk (OR = 2.5) at 95% CI for genotype TC, the lowest relative risk (OR = 0.21) at 95% CI for genotype TT of the same gene. Considering polymorphous variant A-351G of the gene *ESR1*, the largest risk of cancer development (OR at 95% CI is 2.81) is possible at genotype AG of polymorphous variant A-351G of the gene *ESR1*, and a minor risk (0.24) at genotype AA of polymorphous variant A-351G of the gene *ESR1.*

Table 5

The frequency of genotypes of polymorphous variants of the gene *ESR1* (T-397C, A-351G)

Gene *ESR1* Polymorphous variant T-397C	Cancer (n = 90)		Control (n = 55)		Statistical analysis results			
Genotypes	**n**	**%**	**n**	**%**	**χ^2**	**p**	**OR**	**95% CI**
TT	23	25.56	34	61.82	**17.33**	**0.001**	**0.21**	**0.10–0.44**
TC	47	52.22	18	32.73	**4.49**	**0.034**	**2.25**	**1.12–4.52**
CC	20	22.22	3	5.45	**5.99**	**0.014**	**4.95**	**1.40–17.55**
Gene *ESR1* Polymorphous variant A-351G	**Cancer (n =90)**		**Control (n = 55)**		**Statistical analysis results**			
Genotypes	**n**	**%**	**n**	**%**	**χ^2**	**p**	**OR**	**95% CI**
AA	30	33.33	37	67.27	**14.48**	**0.001**	**0.24**	**0.12–0.50**
AG	52	57.78	18	32.73	**7.61**	**0.006**	**2.81**	**1.39–5.67**
GG	8	8.89	0	0.00	3.61	0.057	–*	–

*OR was not calculated due to absence of genotype GG in control.

Table 6

The differences in frequencies of genotypes occurrence of polymorphous variants T-397C, A-351G of the gene *ESR1* in comparison groups — patients with FRSO cancer from families with cancer family history and healthy females

Probands examined, n/%	Genotype of polymorphous variant T-397C and number of patients, n (%)			Genotype of polymorphous variant A-351G and number of patients, n (%)		
	397TT	**397TC**	**397CC**	**351AA**	**351AG**	**351GG**
EC (23/100%)	3 (13.0)	10 (43.5)	10 (43.5)	11 (47.8)	10 (43.5)	2 (8.7)
BC (21/100%)	3 (14.3)	13 (61.9)	5 (23.8)	4 (19.1)	15 (71.4)	2 (9.5)
Uterine body cancer (46/100%)	17 (36.9)	24 (52.2)	5 (10.9)	15 (32.6)	27 (58.7)	4 (8.7)
TOTAL number of FRSO cancer patients, n = 90/100%	23 (25.6)	47 **(52.2)**	20 (22.2)	30 (33.3)	52 **(57.8)**	8 (8.9)
Control group – healthy females, n = 55/100%	34 **(61.8)**	18 (32.7)	3 (5.5)	37 **(67.3)**	18 (32.7)	–

Therefore, the data presented suggest the variability of the risk of oncologic pathology development depending on genotypes of polymorphous variants T-397C and A-351G of the gene *ESR1*. The largest risk of cancer development in females with family cancer syndrome in family was determined in case of genotype CC of polymorphous variant of the gene *ESR1* (T-397C), and 2.2 times lesser risk at genotype TC of the same polymorphous gene variant. In case of polymorphous variant of the gene *ESR1* (A-351G) the largest risk was found at genotype AG.

During analysis of variability of genotypes frequencies a number of issues emerged: what was a frequency of determined polymorphous variants of the gene *ESR1* (T-397C, A-351G) in patients from families with cancer family history and in practically healthy females, what was a frequency of polymorphous variants of the gene *ESR1* depending on tumors genesis — in patients with OC and BC, what was genetic risk of cancer development in families with aggregation of tumor pathology. As it can be seen from the Table 6, in patients with FRSO polymorphous variants of the gene *ESR1* (A-351G) are determined with almost similar frequency — 57.8% of patients, comparing with polymorphous variants of the gene *ESR1* (T-397C) — 52.2% ($p > 0.05$). Healthy females do not have differences between the same polymorphous variants at all, 32.7%, respectively. In contrast, in healthy females more frequently ($p < 0.05$) genotype 397TT (61.8%) of polymorphous variant of the gene *ESR1* (T-397C) and genotype 351AA (67.3%) of polymorphous variant of the gene *ESR1* (A-351G) are determined, comparing with its frequency in cancer patients: 25.6 and 33.3%, respectively.

Therefore, provided complex examination determined the frequency of SNP of receptor gene *ESR1* and assessed the risk of malignant pathology development in families of probands with family cancer syndrome. The obtained data indicate individual genotypes alterations that may be associated both with tumor genesis and with genetic mutation in families that are passed by family members from generation to generation. The obtained data complement and deepen the data of our previous clinical-genealogical examinations and molecular-genetic studies of peripheral blood of females with FRSO cancer [20]. In Western region of Ukraine (Lviv region) at clinical-genetic females examination the authors found mutations in *BRCA1/2* genes in patients with family/hereditary BC [21]. We determined that in predisposition of females of Ukrainian population to FRSO cancer development, in particular, to family/hereditary BC development, not only mutations in genes-suppressors of tumor growth *BRCA1* and *BRCA2* play the role, but also mutations in the gene *ESR1* are involved.

The mechanisms of malignant transformation of somatic cells and predisposition to cancer development — are the most actual issues of oncology. It is known that proliferation of the cells of hormone-dependent organs is estrogen-regulated. Disorders of hormones' physiological influence on cells of breast, ovaries and uterus may occur resulting from alterations of hormones levels or disorder of balance between hormone fractions, or due to the damage of molecular structure of receptor gene *ERS1*. The latter can be caused by mutations, in particular, by single nucleotide substitutions (SNP) in a gene that in turn contributes to disruption of normal pathway of gene *ERS1* activation.

In recent years, the role of SNP in cell malignization has been widely studied and actively discussed. As causal factors of BC 16 candidate SNPs and 13 genes were determined. Among the latter significant attention is paid to gene *MBIP* (MAP3K12-binding inhibitory protein 1) that regulates activity of cycline-dependent kinases and gene *RPS6KA* that participates in control of growth and cell differentiation. The gene *MBIP* product functions as membrane receptor that is involved in different physiological and pathological processes (in embryogenesis, homeostasis, reparation, immune response and tumor cells metastasing). Also several signal pathways of regulation of the gene *PTEN* that makes a definite contribution to predisposition to BC development were determined; its expression reduction is associated with worse BC prognosis [22, 23]. The above is a convincing evidence of the cardinal role of genetic alterations in cancer pathogenesis that does not diminish the importance of epigenetic changes that occur under the influence of intrinsic factors of the organism, influencing on particularities of its development and causing age-dependent diseases [24].

Obviously, for tumor development associations of multiple genes polymorphisms and epigenetic changes are of importance. It was determined that in such cases only one third of associations was statistically linked to genes, coding cellular metabolism [25]. Through meta-analysis significant associations of other genes polymorphism with neoplasias risk were identified. The majority of researchers from different countries consider mutation of one gene allele as a genetic factor of predisposition to BC — heterozygous clones with gene *BRCF1/2* mutation lead to genomic instability that may be genetic background for cells malignization, and mutation in two gene alleles — the reason of tumor genesis [26, 27]. Some authors [28–30] during exploration of germinal mutations in genes concluded that gene polymorphism acted as a genetic modifier of prognosis (better or worse). According to some authors [30] genomic instability — is a «hallmark of cancer», leading to genetic alterations augmentation, tumor genesis and tumor progression. Different types of genetic instability (nucleotide, microsatellite, and chromosome) occur both in individual characteristics, and through different tumors. According to the data of investigation of numerous genomic associations (GWASs) and meta-analysis data [4] there is a wide range of polymorphous gene variants in cancer patients, and only 31.7% of genes of 145 polymorphous gene variants were associated with BC. Studies on relation of gene mutations to receptor status of breast cells will be interesting. It was demonstrated that, depending on BC receptor status, different mutations of the genes *BRCA1/2* are determined: increased frequency of C homozygotes in patients with estrogen-negative tumors, whereas in other situation (positive estrogen and progesterone receptor status) other mutations were observed.

In general, the knowledge of occurrence of polymorphous variants of receptor gene *ESR1,* associated with increase of neoplasias risk, is extremely important at medical-genetic counseling and examination of females, in particular, in females from families with cancer family history for determination of potential risk of neoplasias development. Adequate prognosis for the development of FRSO cancer and OC in females with tumor pathology in several generations of their family trees is impossible without knowledge of family cancer history and studies of polymorphism of estrogen gene *ERS1* in such females. Provided studies and their continuation is an important first step and one of predictive genomics approaches. In terms of research performed — this is a determination of individual genetic alterations in tumor cells and individual risk of malignization development in persons, having cancer patients in their pedigrees.

Our results open the perspective of novel strategy for further studies of the problem of early diagnostics and prevention of FRSO malignant tumors. Its focus lies not only in identifying families with aggregation of malignant tumors in their pedigrees, but also in identification of carriers of mutations of genes-suppressors *BRCA1* and *BRCA2* and SNP of receptor gene *ESR1* in families with family cancer syndrome or PMT. Establishment of clinical-genetic register of such persons within certain regions of Ukraine to address issues concerning monitoring of their health, prophylaxis 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 examination of patients with BC, OC determined wide range of tumor pathology; most of them were patients with CC (31.5%). Total number of cancers of hormone-dependent organs (uterine body cancer, BC, OC) was 62.3%. Tumors association in family trees corresponds to family cancer syndrome (Lynch II syndrome).

2. In patients the frequency of genotype TT of polymorphous variant of the gene *ESR1* (T-397C) was significantly lower than in control and comprised 25.56% *versus* 61.82%. In contrast, the frequency of genotypes TC (52.22%) and CC (22.22%) of the same polymorphous variant of the gene *ESR1* was significantly higher comparing with control data — 32.73% and 5.45%, respectively.

3. The frequency of genotypes AA, AG, GG of the gene *ESR1* (A-351G) also was different in cancer patients comparing with these values in healthy females: significant reduction of genotype AA frequency to 33.33% *versus* 67.27% in healthy females, significant increase of genotype AD frequency from 32.73% to 57.78%, tendency to increase of genotype GG frequency — from zero to 8.89%.

4.In patients with FRSO cancer significant changes in the frequency of polymorphous variants of the gene *ESR1* (A-351G) were not found — (57.8% of patients) and polymorphous variants of the gene *ESR1* (T-397C) — (52.2% of patients). In healthy females the difference between the same polymorphous variants of the gene *ESR1* also was not seen (32.7%, respectively). In contrast, in healthy females genotype 397TT (61.8%)

of polymorphous variant of the gene *ESR1* (T-397C) and genotype 351AA (67.3%) of polymorphous variant of the gene *ESR1* (A-351G) was detected more frequently ($p < 0.05$) comparing with their frequency in cancer patients: 25.6 and 33.3%, respectively.

5. The risk of tumor development (OR; 95% CI) in probands' families with cancer aggregation depends on polymorphous variant of the gene *ESR1*. The highest OR is 4.95 (1.40–17.55) at genotype CC of polymorphous variant of the gene *ESR1* (T-397C), whereas at genotype TC of the same polymorphous variant of the gene it reduces to 2.25 (1.12–4.52). OR for 95% CI at polymorphous variant of the gene *ESR1* (A-351G) is lower and it comprises 2.81 (1.39–5.67). For genotype 351AA of the same gene protective effect was estimated.

6. The obtained results suggest the need for clinical-genealogical and molecular-biological studies in patients with FRSO cancer with burdened family history of malignant pathology for formation of genetic risk groups and monitoring of females health, for cancer prevention by treatment of pre-cancerous and inflammatory FRSO processes. Determination of SNPs of estrogen receptor gene *ESR1* as genetic markers of predisposition to malignant tumors development is a basis for their application at development of clinical protocols of examination of members of families with family cancer syndrome for determination of individual risk of neoplasias development.

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КЛІНІЧНЕ ЗНАЧЕННЯ МУТАЦІЙ (SNP) ГЕНА РЕЦЕПТОРА ЕСТРОГЕНА *ERS1* У ХВОРИХ НА РАК З ОНКОЛОГІЧНОЮ ПАТОЛОГІЄЮ У РОДОВОДАХ

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Резюме. *Стаття присвячена визначенню генетичного ризику виникнення онкологічної патології у родинах пробандів із сімейною історією раку (пробанди — хворі на рак молочної залози, рак яєчника, первинно-множинні пухлини) шляхом оцінки результатів клініко-генеалогічного обстеження хворих та генетичного аналізу мутацій (SNP — однонуклеотидний поліморфізм) гена рецептора естрогена ERS1.* ***Методи дослідження:*** *клінічний, морфологічний, клініко-генеалогічний, молекулярно-генетичний, статистичний.* ***Результати:*** *серед обстежених було 90 хворих на рак і 55 здорових жінок без випадків раку у родоводах (група контролю). Встановлено, що асоціація злоякісних пухлин у родоводах хворих жінок відповідала сімейному раковому синдрому (синдром Лінча II типу). При молекулярно-генетичному дослідженні геномної ДНК периферичної крові та гістологічного матеріалу пухлин визначено достовірне підвищення частоти генотипу 397CC гена ESR1 у хворих на рак з агрегацією злоякісної патології у родинах порівняно з частотою цього генотипу у жінок із групи контролю. Статистичний аналіз показав, що ризик розвитку злоякісної патології (відношення шансів — ВШ; 95% довірчий інтервал — ДІ) підвищується у 4,95 раза у випадках CC генотипу гена ESR1 (T-397C), а у разі гетероносійства цього гена — у 2,25 раза. За наявності TT генотипу гена ESR1 (T-397C) ризик розвитку раку знижується (ВШ 0,21).* ***Висновок:*** *частота генотипів AA, AG, GG гена ESR1 (A-351G) також відрізняється у хворих на рак порівняно зі здоровими жінками: значне зниження частоти AA генотипу до 33,33% порівняно з 67,27% у здорових жінок (p = 0,001), значне підвищення частоти AG генотипу із 32,73 до 57,78% (p = 0,006), тенденція до зростання частоти GG генотипу — від 0 до 8,89% (p = 0,057). Одержані результати є основою для проведення клініко-генеалогічних та молекулярно-генетичних досліджень у родинах з агрегацією раку органів жіночої репродуктивної системи для детекції SNP гена ESR1. Виявлений SNP поліморфізм вказаного гена може бути предиктором розвитку раку та агрегації злоякісних пухлин у родинах пробандів та їх нащадків.*

Ключові слова: пухлини органів жіночої репродуктивної системи, родовід, сімейний раковий синдром, однонуклеотидний поліморфізм гена *ESR1*.

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