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ABSTRACT

Natalia Tsyndrenko
https://orcid.org/0000-0001-6763-476X
Department of Pathology, Sumy State University, Sumy, Ukraine

Anatoliy Romaniuk
https://orcid.org/0000-0003-2560-1382
Department of Pathology, Sumy State University, Sumy, Ukraine

PVUII (RS2234693) POLYMORPHISM OF THE ESTROGEN RECEPTOR ALPHA GENE IN WOMEN FROM SUMY OBLAST, UKRAINE, WITH ENDOMETRIAL HYPERPLASTIC PROCESS

Introduction. The endometrial hyperplastic process is an estrogen-dependent benign condition of the uterus, which is frequently a cause of infertility, ovarian-menstrual cycle disorders, and malignant transformation into uterine cancer. The effect of estrogen on the endometrium is realized through the estrogen receptor alpha. It is manifested by a whole range of biological changes, including cell reproduction and growth, tissue development, etc. Estrogen receptor alpha is encoded by the ESR1 gene, which is located on chromosome 6q25 and has eight exons and seven introns. Today, about 9,000 polymorphisms of the ESR1 gene have been described, some of which are associated with gene activity changes. Such functional polymorphisms include the PvuII polymorphism (rs2234693), which occurs due to the substitution of thymine (T) for cytosine (C) in the first intron of the gene.

Objective. The purpose of this study was to assess the frequency of allelic variants of the estrogen receptor alpha gene for the PvuII polymorphism in patients with endometrial hyperplastic process living in the Sumy Oblast of Ukraine and to study its relationship with the histological variant of endometrial hyperplastic process, anthropometric parameters, age categories, medical history, and concomitant diseases.

Materials and Methods. Genomic DNA was isolated from blood samples of 95 women with endometrial hyperplastic processes and 80 healthy women. The rs2234693 polymorphism was studied using a polymerase chain reaction with subsequent restriction fragment length polymorphism analysis (PCR-RFLP). The data were processed and statistically analyzed with Microsoft Excel and SPSS Statistics 29.0 for Windows software package. Descriptive statistics, Student's test,

ANOVA method, and Pearson's chi-squared test were used in this study. Results with $P < 0.05$ were considered statistically significant.

Results. The distribution of genotype variants for the PvuII polymorphism of the ESR1 gene in women with endometrial hyperplastic process was: homozygotes for the major allele (T/T) – 31.6%, heterozygotes (T/C) – 49.5%, homozygotes for the minor allele (C/C) – 18.9%; in the control group, these values were 30%, 52.5%, and 17.5%, respectively. There was no difference in the distribution of genotypes in patients with endometrial hyperplastic processes and healthy women ($\chi^2 = 0.163$, $P = 0.922$). No associations were found between the PvuII polymorphism and the histological variant of endometrial hyperplastic processes ($\chi^2 = 4.14$, $P = 0.387$), anthropometric parameters ($P > 0.05$), age ($\chi^2 = 2.98$, $P = 0.560$), medical history ($P > 0.05$), or concomitant genital and extragenital conditions ($P > 0.05$).

Conclusions. There was no difference in the distribution of T/T, T/C, and C/C genotypes for the PvuII polymorphism in the estrogen receptor alpha gene between patients with endometrial hyperplastic process and the control group. There was no correlation between the genotype variant for the studied polymorphism and the histological variant of the endometrial hyperplastic process, anthropometric parameters, age, medical history, concomitant genital and extragenital conditions in patients of the Sumy Oblast, Ukraine.

Keywords: estrogen receptor alpha gene, endometrial hyperplastic process, PvuII polymorphism, endometrial polyp.

Corresponding author: *Natalia Tsyndrenko, Department of Pathology, Sumy State University, Sumy, Ukraine*
e-mail: nebesenko.n@ukr.net

РЕЗЮМЕ

Наталія Циндренко
https://orcid.org/0000-0001-6763-476X
Кафедра патологічної анатомії Сумського державного університету, м. Суми, Україна

Анатолій Романюк
https://orcid.org/0000-0003-2560-1382
Кафедра патологічної анатомії Сумського державного університету, м. Суми, Україна

ПОЛІМОРФІЗМ PVUII (RS2234693) ГЕНА РЕЦЕПТОРА ЕСТРОГЕНУ АЛЬФА У ЖІНОК СУМСЬКОГО РЕГІОНУ УКРАЇНИ З ГІПЕРПЛАСТИЧНИМИ ПРОЦЕСАМИ ЕНДОМЕТРІЯ

Вступ. Гіперпластичні процеси ендометрія – естрогензалежні доброякісні патології матки, які є частою причиною неплідддя, порушень овариально-менструального циклу та малігнізації у рак тіла матки. Дія естрогену на ендометрій реалізується через рецептор естрогену альфа і проявляється цілим спектром біологічних змін, включаючи розмноження і ріст клітин, розвиток тканин та інші. Рецептор естрогену альфа кодується геном ESR1, який розташований на хромосомі 6q25 і має вісім екзонів та сім інтронів. Сьогодні описано близько 9 тисяч поліморфізмів гена ESR1, частина з яких пов'язана зі зміною його активності. Серед таких функціональних поліморфізмів – поліморфізм PvuII (rs2234693), який виникає внаслідок заміни тиміну (T) на цитозин (C) у першому інтроні гена.

Мета. Метою даного дослідження була оцінка частоти алельних варіантів гена рецептора естрогену альфа за PvuII поліморфізмом у пацієнток з гіперпластичними процесами ендометрія, що мешкають у Сумському регіоні України та вивчення його зв'язків з гістологічним варіантом гіперпластичних процесів ендометрія, антропометричними

показниками, віковими категоріями, даними анамнезу та супутньою патологією жінок з гіперпластичними процесами ендометрія.

Матеріали та методи. Геномна ДНК була виділена із зразків крові 95 жінок з гіперпластичними процесами ендометрія та 80 жінок без гіперпластичних процесів ендометрія. Дослідження rs2234693 поліморфізму здійснено методом полімеразної ланцюгової реакції з наступним аналізом довжини рестрикційних фрагментів (PCR-RFLP). Математичну обробку та статистичний аналіз даних виконували за допомогою Microsoft Excel та програмного пакета «SPSS Statistics 29.0 for Windows». Використовувалися дискриптивна статистика, критерій Стьюдента, методика ANOVA, критерій χ2 Пірсона. Статистично значущими вважалися результати з $P < 0,05$.

Результати дослідження. Розподіл варіантів генотипів за PvuII поліморфізмом гена ESR1 у жінок з гіперпластичними процесами ендометрія становив: гомозиготи за основним алелем (T/T) – 31,6 %, гетерозиготи (T/C) – 49,5 %, гомозиготи за мінорним алелем (C/C) – 18,9 %; у жінок без гіперпластичних процесів ендометрія відповідно 30 %, 52,5 %, 17,5 %. Різниці у розподілі генотипів у пацієнток із з гіперпластичними процесами ендометрія і жінок без гіперпластичних процесів ендометрія не виявлено ($\chi^2 = 0,163$, $P = 0,922$). Не виявлено асоціацій між PvuII поліморфізмом та гістологічним варіантом гіперпластичних процесів ендометрія ($\chi^2 = 4,14$, $P = 0,387$), антропометричними показниками пацієнтів ($P > 0,05$), віковими групами ($\chi 2 = 2,98$, $P = 0,560$), даними анамнезу ($P > 0,05$), супутньою генітальною та екстрагенітальною патологіями ($P > 0,05$).

Висновки. Відсутня різниця у розподілі генотипів T/T, T/C, C/C за PvuII поліморфізмом гена рецептора естрогену альфа у пацієнток Сумського регіону України, що мають гіперпластичні процеси ендометрія та жінок без гіперпластичних процесів ендометрія. Немає залежності між варіантом генотипу за вивченим поліморфізмом і гістологічним варіантом гіперпластичних процесів ендометрія, антропометричними показниками, віковими групами, даними анамнезу, супутньою генітальною та екстрагенітальною патологіями у пацієнтів.

Ключові слова: ген рецептора естрогену альфа, гіперпластичні процеси ендометрія, поліморфізм PvuII, поліп ендометрія.

Автор, відповідальний за листування *Наталія Циндренко, аспірант кафедри патологічної анатомії Сумського державного університету, м. Суми, Україна*
e-mail: nebesenko.n@ukr.net

INTRODUCTION / ВСТУП

The endometrial hyperplastic process (EHP) is one of the most common gynecological diseases in the world, which affects the lives of many women [1]. EHPs are precursors of endometrioid adenocarcinoma, which is one of the most common malignant tumors of the female genital organs in industrialized countries[2]. EHP is characterized by the proliferation of endometrial glands, which leads to an increased gland-to-stroma ratio [3, 4, 5, 6]. EHP is the result of continuous estrogen stimulation of endometrial tissues with a relative lack of progesterone-balancing effects [7].

The action of estrogen is mediated through its binding to estrogen receptors (ER). ERs have two subtypes – ER-α and ER-β, encoded by estrogen

receptor α (ESR1) and β (ESR2) genes, respectively. They belong to the superfamily of nuclear receptors [8]. Although the two ER isoforms differ in structure, they share similar homology in the DNA and ligand-binding domains. Both ER isoforms are expressed in osteoblasts, osteoclasts, bone marrow, and uterus [9].

The ESR1 gene, as a steroid hormone receptor gene, is located on chromosome 6 at the locus 6p25. The ESR1 gene consists of eight exons and seven introns. In the first intron, two common site polymorphisms are located in restriction enzyme recognition sites, one of which is PvuII (rs2234693). The PvuII single nucleotide polymorphism is the result of the substitution of thymine (T) nucleotide for cytosine (C) (T397C) in the first intron of the gene. Changes in the ESR1 gene cause disruption and loss of receptor sensitivity to hormones [10, 11, 12].

Mutation of the ESR1 gene can induce cell proliferation, regulate cell apoptosis, and affect protein expression, thus participating in the development and progression of tumorigenesis [13]. Different impact of estrogenic effects, different levels of estrogenic molecules, or genetic variability in response to the estrogenic environment may play a key role in tumorigenic transformation of cells [14]. Biallelic and multiallelic polymorphisms were detected in the ESR1 gene and used for the association studies. Unlike mutations, polymorphisms are not directly associated with a specific disease, but they are an important tool in the study of multifactorial disorders [15].

Multiple single nucleotide polymorphisms in the ESR1 gene located in intron 1, including rs2234693, have been studied in many clinical studies and have become a hot topic regarding tumor susceptibility [16]. The PvuII single nucleotide polymorphism of the ESR1 gene has been found to be associated with numerous carcinomas, including prostate cancer, as well as systemic lupus erythematosus, Alzheimer's disease, etc. [17]. The PvuII polymorphism of the ESR1 gene may be associated with the risk of endometrial cancer [18].

Thus, genetic disorders, including polymorphisms and variations in the expression levels of genes that encode estrogen receptors, can cause an impairment of estrogen function and, as a result, lead to EHP [19].

Some studies focused on the association between the PvuII polymorphism of the ESR1 gene and various diseases, including breast cancer, endometrial cancer, uterine leiomyoma, endometriosis, osteoporosis, and others. However, study results are inconsistent, so more research is needed to understand the impact of this polymorphism on disease development. Since there are no studies on the role of the PvuII polymorphism of the ESR1 gene in the susceptibility to EHP among women of the Sumy Oblast of Ukraine, we evaluated this association, as well as the peculiarities of the gynecological history of patients with EHP who had different genotypes for the PvuII polymorphism of the ESR1 gene. Given that EHP (especially atypical EHP and endometrial cancer) is often associated with obesity or excess body weight, hypertension, and diabetes, we investigated their association with the PvuII polymorphism of the ESR1 gene in women with EHP.

Materials and Methods

95 patients diagnosed with EHP (endometrial hyperplasia or endometrial polyp), which was histologically confirmed after surgical treatment (hysteroresectoscopy) (HRS), took part in the study. All patients were treated at the Sumy Regional Clinical Oncology Center (Sumy, Ukraine) from 2020 to 2022. The control group consisted of 80 women without EHP who had preventive checkups at the Municipal Non-Profit Enterprise "Blessed Virgin Mary Clinical Maternity Hospital" of Sumy Municipal Council and Municipal Non-Profit Enterprise "Clinical Hospital No. 4" of Sumy Municipal Council (Sumy, Ukraine) during 2021–2023. The research was conducted in compliance with the fundamental ethical principles of the Declaration of Helsinki of the World Medical Association on conducting scientific medical research involving human subjects. All patients signed the written informed consent for histological examination, blood sample collection, and personal data processing.

Histological samples obtained from women receiving hormones, nonsteroidal anti-inflammatory drugs, and antihormonal drugs were excluded from the study, as well as samples of patients who refused to participate in the study.

In order to study the PvuII polymorphism of the ESR1 gene, venous blood was collected in patients with EHP and healthy subjects under sterile conditions using monovettes (2.7 ml) containing ethylenediaminetetraacetic acid (EDTA) potassium salt (11.7 mM) as an anticoagulant ("Sarstedt", Germany). Samples were frozen and stored at -20 ºC.

DNA was isolated from whole blood leukocytes using GeneJET Whole Blood Genomic DNA Purification Mini Kit (USA). The method is based on

the reversible binding of nucleic acids using a silica-based membrane in a spin column. Blood samples were lysed in the lysing buffer containing proteinase K. Next, the lysate was mixed with ethanol and placed in the spin column, where the DNA reversibly binds to a silica membrane. Impurities were effectively removed by washing the spin column with prepared washing buffers. Genomic DNA was then eluted under low ionic strength conditions with an elution buffer. Pure DNA yield from 200 mcL of whole blood equaled 2–10 mcg. In the process of DNA extraction, we followed the recommendations provided in the commercial kit and performed procedures according to the protocol.

The PvuII polymorphism (rs2234693) in intron 1 of the ESR1 gene was identified using polymerase chain reaction with subsequent analysis of restriction fragment length polymorphism (PCR-RFLP). The gene region was amplified using a pair of specific primers: a direct one (sense) – 5' CACACATCACCATTCTCAGC 3' and a reverse one (antisense) – 5' TCTAGACCACACTCAGGGTCTC 3'. The primers were synthesized by Metabion (Germany). For amplification, 50–100 ng of DNA was taken and added to the mixture containing 5 mcL of 5x PCR buffer, 1.5 mM of magnesium sulfate, 200 μm of a mixture of four nucleotide triphosphates, 15 pM of each primer, and 0.75 U of Taq-polymerase ("Thermo Scientific", USA); the volume was adjusted to 25 mcL with deionized water. PCR was performed using GeneAmp PCR System 2700 thermal cycler ("Applied Biosystems," USA). Amplification of the fragment of intron 1 consisted of 33 cycles: denaturation – 94 °C (50 s), primer hybridization – 64.5 °C (45 s), and elongation – 72 °C (1 min). Then, 6 mcL of the amplified product was incubated at 37 °C for 18 hours with 3 U of PvuII restriction enzymes ("Thermo Scientific," USA) in buffer R consisting of: 10 mM of tris HCl (pH 7.5), 10 mM of magnesium chloride, 50 mM of NaCl, and 0.1 mg/ml of albumin. If thymine was in position 647 in the ESR1 gene, the amplificate, consisting of 392 base pairs (bp), was digested with PvuII restriction enzyme, producing two fragments: 342 and 50 base pairs. In the case of thymine to cytosine substitution, the PvuII restriction site loss occurred, and one fragment of 392 base pairs was formed. After restriction, the amplificates were separated on 2.5% agarose gel containing 10 μg/mL of ethidium bromide. Horizontal electrophoresis (0.13 A; 200V) was performed for 35 min. After electrophoresis, DNA visualization was carried out using a Biocom transiluminator.

The data were processed using standard methods, such as Microsoft Excel. To evaluate the obtained results, they were statistically analyzed with the SPSS Statistics 29.0 for Windows software package. Samples were characterized by descriptive statistics. The arithmetic mean (M) of the variation series and the standard deviation (SD) were calculated (data are presented as $M \pm SD$). The Student's test (t) was used to compare the values of two independent samples with the normal distribution. The statistical significance of the difference among the three groups was assessed using one-way analysis of variance (ANOVA). Pearson's chi-squared test was used to determine the statistical significance of the difference in allele and genotype frequencies among groups of patients. Results with $P < 0.05$ were considered statistically significant.

Results

Frequency of PvuII polymorphism of the ESR1 gene in patients with EHP and the control group

In accordance with the set goal, we carried out genotyping for the PvuII polymorphism of the ESR1 gene in patients with EHP and healthy women; the frequency of T and C alleles was determined, as well as the ratio between the major allele homozygotes (T/T), heterozygotes (T/C), and the minor allele homozygotes (C/C).

The distribution of genotypes for the studied polymorphism did not deviate from the Hardy-Weinberg equilibrium ($P > 0.05$) in the main or control group. T and C allele frequencies were equivalent in the group of patients with EHP and in healthy women and amounted to 0.56 and 0.44, respectively. At the same time, the indicator of statistical significance (P) was equal to 1. This indicates the absence of difference in the distribution of alleles between subjects of the comparison groups.

T/T : T/C : C/C genotype ratio in patients with EHP was 31.6% : 49.5% : 18.9%, and in the group of healthy women it was 30% : 52.5% : 17.5%. Based on the results of Pearson's chi-squared test, the difference between these indicators was not statistically significant ($P = 0.922$). Therefore, we could assume that there was no association between the PvuII allelic polymorphism of the ESR1 gene and the development of EHP (Table 1).

Table 1 – Distribution of alleles and genotypes for the PvuII polymorphism of the ESR1 gene in women with EHP and the control group

	Patients with EHP	**Control group**
T-allele	0.56	0.56
C-allele	0.44	0.44
$\chi^2 = 2$; P = 1		
T/T homozygotes, n (%)	30 (31.6)	24 (30)
T/C heterozygotes, n (%)	47 (49.5)	42 (52.5)
C/C homozygotes, n (%)	18 (18.9)	14 (17.5)
Total	95 (100)	80 (100)
$\chi^2 = 0.163$; P = 0.922		
P'	> 0.05	> 0.05

Note: n = number of patients, P = statistically significant difference between the main and control groups; P' = deviation from the Hardy-Weinberg equilibrium in each group

Patient groups characteristics

When analyzing the data, we found that the average age of women with EHP was 48.25 ± 11.8 years. At the same time, women in the control group were significantly older (69.93 ± 8.53) (t = -14.115, P < 0.001) vs. women in the main group. The average height of women in the main group was 164.68 ± 6.10 cm, which hardly differed from that of women in the control group (163.13 ± 4.82) (t = 1.807, P = 0.071). The average weight of women in the main group amounted to 71.72 ± 14.81 and was greater than that of women in the control group (67.37 ± 6.21) (t = 2.0; P = 0.044). The average BMI in the main group was slightly higher than in the control group – 26.68 ± 5.82, but we did not observe a significant difference in BMI values between the groups (t = 1.302; P = 0.201) (Table 2). All examined women in both groups were ethnic Ukrainians.

Table 2 – General clinical characteristics of patients with EHP and the control group

Parameter	**Patients with EHP (n = 95)**	**Control group (n = 80)**	**P**
Age, years	48.25 ± 11.81	69.93 ± 8.53	**<0.001**
Age groups:			
reproductive age (< 45 years)	46 (48.4%)	-	**<0.001**
premenopausal age (45–55 years)	21 (22.1%)	-	
menopausal age (> 56 years)	28 (29.5%)	80 (100%)	
Body weight, kg	71.72 ± 14.81	67.37 ± 6.21	**0.044**
Height, cm	164.68 ± 6.10	163.13 ± 4.82	0.071
BMI, kg/m^2	26.68 ± 5.82	25.78 ± 2.38	0.201

Note: n = number of patients; BMI = body mass index; P = statistically significant difference according to t-test results

The correlation between morphology and genotypes for the PvuII polymorphism of the ESR1 gene in EHP patients

According to the pathohistological report, the studied group of women with EHP included 29 cases of atypical endometrial hyperplasia (EH) (30.5%), 11 cases of glandular endometrial polyps (GEP) (11.6%), and 55 cases of glandular-fibrous endometrial polyps (GFEP) (57.9%). Using Pearson's chi-squared test, we showed that the

histological variant of EHP does not depend on the genotype of the PvuII polymorphism of the ESR1 gene (χ^2 = 4.14; P = 0.387) (Table 3).

The correlation between the PvuII allelic polymorphism of the ESR1 gene and the development of EHP with regard to anthropometric parameters and age of subjects

When analyzing the data, we found that the average age of women with EHP was 48.25 ± 11.8 years. At the same time, no statistically significant difference was found in age between groups of women with different genotypes for the PvuII polymorphism of the ESR1 gene (F = 1.24, P = 0.293). The average height of women in the main group was 164.68 ± 6.10 cm; it hardly differed in groups with different genotypes for the PvuII polymorphism of the ESR1 gene (F = 0.39, P = 0.679). The average weight in the main group amounted to 71.72 ± 14.81 kg. No statistically significant difference was found for this parameter between the groups (F = 1.92, P = 0.153). The average BMI in the main group was 26.68 ± 5.82. We did not find a significant difference in BMI values among subjects with different genotypes for the PvuII polymorphism of the ESR1 gene (F = 1.00; P = 0.371). It should be noted that female patients were overweight or obese in 46.3% of cases (Table 4).

Table 3 – Dependence of the EHP histological variant on the genotype for the PvuII polymorphism of the ESR1 gene

Genotype	**EHP histological variant, n (%)**			**Total, n (%)**
	Non-atypical EH	GEP	GFEP	
T/T	6 (6.3)	5 (5.3)	19 (20.0)	30 (31.6)
T/C	17 (17.9)	3 (3.2)	27 (28.3)	47 (49.5)
C/C	6 (6.3)	3 (3.2)	9 (9.5)	18 (18.9)
Total, n (%)	29 (30.5)	11 (11.6)	55 (57.9)	95 (100)
χ^2 = 4.14; P = 0.387				

Note: n = number of patients; P = statistically significant difference according to chi-squared test results

Table 4 – Anthropometric parameters in patients with EHP depending on the genotype for the Pvull polymorphism in the ESR1 gene (M ± SD)

Parameter	**T/T**	**T/C**	**C/C**	**F**	**P**
	n = 30	*n = 47*	*n = 18*		
Age	46.77 ± 10.94	50.15 ± 12.40	45.78 ± 11.45	1.24	0.293
Height, cm	163.87 ± 5.72	165.04 ± 6.52	165.11 ± 5.77	0.39	0.679
Body weight, kg	68.23 ± 15.69	74.64 ± 14.17	69.89 ± 14.20	1.92	0.153
BMI, kg/m^2	25.66 ± 6.15	27.51 ± 5.62	26.20 ± 5.77	1.00	0.371

Note: n = number of patients; P = statistically significant difference according to F-test results (ANOVA method)

Given that age is a significant risk factor for reproductive system diseases, particularly EHP, we investigated the association of allelic variants of the ESR1 gene for the PvuII polymorphism with age. All patients were divided into three age groups. The first group (under 45 years) included 46 (48.4%) individuals, the second group (45–55 years) included 21 individuals (22.1%), and the third group (over 55 years) included 28 individuals (29.4%), respectively. The distribution of the ESR1 gene allelic variants for the PvuII polymorphism did not differ significantly in the age groups (χ^2 = 2.98; P = 0.560) (Table 5).

Table 5 – Frequency of allelic variants of the ESR1 gene for the PvuII polymorphism in patients with EHP in different age groups

Genotype	Age, n (%)			Total, n (%)
	Up to 45 years	45–55 years	Older than 55	
T/T	15 (15.8)	8 (8.4)	7 (7.4)	30 (31.6)
T/C	20 (21.1)	11 (11.6)	16 (16.8)	47 (49.5)
C/C	11 (11.6)	2 (2.1)	5 (5.3)	18 (18.9)
Total, n (%)	46 (48.4)	21 (22.1)	28 (29.4)	95 (100)
$\chi^2 = 2.98$; P = 0.560				

Note: n = number of patients; P = statistically significant difference according to chi-squared test results

Gynecological status of women with EHP depending on the genotype variant of the Pvull polymorphism of the ESR1 gene

It is well known that various diseases leading to reproductive impairment in women can increase the risk of both malignant and benign neoplasms. In our study, we investigated the peculiarities of the gynecological anamnesis in patients with EHP who had different genotypes for the PvuII polymorphism of the ESR1 gene (Table 6).

Table 6 – Distribution of allelic variants of the ESR1 gene for the PvuII polymorphism in patients with EHP with regard to gynecological parameters

Gynecological parameter		Genotype, n (%)			P by χ^2
		T/T	T/C	C/C	
Age at menarche	< 15 years	27 (28.4)	42 (44.2)	2 (2.1)	$\chi^2 = 0.41$ P = 0.816
	> 15 years	3 (3.2)	5 (5.3)	1 (1.1)	
Parity	No	4 (4.2)	8 (8.4)	2 (2.1)	$\chi^2 = 0.43$ P = 0.806
	Yes	26 (27.4)	39 (41.1)	16 (16.8)	
Artificial abortion	No	17 (17.9)	23 (24.2)	12 (12.6)	$\chi^2 = 1.72$ P = 0.424
	Yes	13 (13.7)	24 (25.3)	6 (6.3)	
Miscarriage	No	26 (27.4)	35 (36.8)	17 (17.9)	$\chi^2 = 4.16$ P = 0.125
	Yes	4 (4.2)	12 (12.6)	1 (1.1)	
History of EHP	No	20 (21.1)	32 (33.7)	11 (11.6)	$\chi^2 = 0.290$ P = 0.867
	Yes	10 (10.5)	15 (15.8)	7 (7.4)	
History of diagnostic scrapings or hysteroresectoscopy	No	22 (23.2)	33 (34.7)	11 (11.6)	$\chi^2 = 0.82$ P = 0.665
	Yes	8 (8.4)	14 (14.7)	7 (7.4)	
Gynecological surgeries	No	22 (23.2)	29 (30.5)	15 (15.8)	$\chi^2 = 3.18$ P = 0.204
	Yes	8 (8.4)	18 (18.9)	3 (3.2)	
Concomitant uterine leiomyoma	Yes	5 (5.3)	19 (20.0)	5 (5.3)	$\chi^2 = 4.95$ P = 0.084
	No	25 (26.3)	28 (29.5)	13 (13.7)	
Concomitant genital endometriosis	Yes	13 (13.7)	15 (15.8)	4 (4.2)	$\chi^2 = 2.37$ P = 0.305
	No	17 (17.9)	32 (33.7)	14 (14.7)	
Burdened familial history	No	25 (26.3)	40 (42.1)	16 (16.8)	$\chi^2 = 0.28$ P = 0.870
	Yes	5 (5.3)	7 (7.4)	2 (2.1)	

Note: n = number of patients; P = statistically significant difference according to chi-squared test results

Calculation of data using the Pearson's chi-squared test showed no correlation between the age at menarche (χ^2 =0.41; P = 0.816), parity (χ^2 = 0.43; P = 0.806), history of artificial abortions (χ^2 = 1.72; P = 0.424), miscarriages (χ^2 = 4.16; P = 0.125), diagnostic scrapings or hysteroresectoscopy (χ^2 = 0.82; P = 0.665), gynecological surgeries (χ^2 = 3.18; P = 0.204) and the studied ESR1 gene polymorphism.

When studying the distribution of allelic variants for the PvuII polymorphism of the ESR1 gene in women with EHP who had a recurrent course of the disease and in women with EHP who had no history of EHP, we found no statistically significant difference between these two groups (χ^2 = 0.290; P = 0.867).

Since the presence of combined hyperproliferative processes of the female genital organs affects the reproductive function as well as the course and effectiveness of treatment, the next step was to study the correlation between a concomitant gynecological disease (uterine leiomyomas, endometriosis) and the genotype for the PvuII polymorphism of the ESR1 gene in women with EHP. Among the subjects, 29 women (30.5%) had uterine leiomyoma, and 32 women (33.7%) had genital endometriosis. However, Pearson's chi-squared test did not reveal any association of uterine leiomyoma and genital endometriosis with the PvuII polymorphism of the ESR1 gene. The difference in the distribution of different allelic variants was not statistically significant (χ^2 = 4.95; P = 0.084 and χ^2 = 2.37; P = 0.305 for leiomyoma and endometriosis, respectively).

When assessing burdened familial history in relation to malignant tumors of the female genital organs, no statistically significant difference was found between the groups for this parameter (χ^2 = 0.28; P = 0.870).

The division of patients into subgroups according to the age at menarche did not reveal any correlation between the genotype distribution for the PvuII polymorphism of the ESR1 gene and the risk of EHP both in patients with menarche occurrence before 15 years and in women with menarche occurrence after 15 years (P1 > 0.05). At the same time, menarche more often occurred before 15 years in patients with EHP vs. the control group (90.4% and 61.2%, respectively). No statistically significant difference was found in the frequency of genotypes between the compared subgroups and among the subjects within the control and main groups (P2 = 0.252; P3 = 0.816) (Table 7).

Table 7 – Genotype distribution for the PvuII polymorphism of the ESR1 gene in the control group and in patients with EHP with regard to the age at menarche

Genotype	Age at menarche before 15 years, n (%)		Age at menarche after 15 years, n (%)	
	Control group	Patients with EHP	Control group	Patients with EHP
T/T	18 (22.5)	27 (28.4)	6 (7.5)	3 (3.2)
T/C	23 (28.7)	42 (44.1)	19 (23.8)	5 (5.3)
C/C	8 (10.0)	17 (17.9)	6 (7.5)	1 (1.1)
	χ^2 = 6.0; P1 = 0.199		χ^2 = 3.0; P1 = 0.223	
P2 = 0.252; P3 = 0.816				

Note: n = number of patients; P1 = statistically significant difference between the control group and the main group according to chi-squared test results; P2 = statistically significant difference among women of the control group; P3 = statistically significant difference among patients with EHP

Extragenital diseases in women with EHP depending on the genotype variant of the Pvull polymorphism of the ESR1 gene

Obesity, essential hypertension, and diabetes mellitus are independent risk factors for the development of EHP and endometrial cancer. We studied their association with the PvuII polymorphism of the ESR1 gene in women with EHP. As shown in Table 6, the frequency of different allelic variants of the ESR1 gene for the PvuII polymorphism did not differ significantly in the studied groups with respect to obesity, essential hypertension, and diabetes. The difference in the distribution of different allelic variants was not statistically significant (χ^2 = 0.67, P = 0.716; χ^2 = 0.67, P = 0.714; and χ^2 = 0.46, P = 0.795, respectively) (Table 8).

Table 8 – Distribution of allelic variants of the ESR1 gene for the PvuII polymorphism in patients with EHP with regard to extragenital diseases

Extragenital disease		Genotype, n (%)			P by χ^2
		T/T	T/C	C/C	
Obesity	Yes	7 (7.4)	15 (15.8)	5 (5.3)	$\chi^2 = 0.67$ P = 0.716
	No	23 (24.2)	32 (33.7)	13 (13.7)	
Essential hypertension	Yes	5 (5.3)	11 (11.6)	3 (3.2)	$\chi^2 = 0.67$ P = 0.714
	No	25 (26.3)	36 (37.9)	15 (15.8)	
Diabetes mellitus	Yes	3 (3.2)	3 (3.2)	1 (1.1)	$\chi^2 = 0.46$ P = 0.795
	No	27 (28.4)	44 (46.3)	17 (17.9)	

Note: n = number of patients; P = statistically significant difference according to chi-squared test results

Discussion

Exploring genetic components plays an important role in studying the pathogenesis of multifactorial diseases, including benign diseases of the female reproductive system. It is known that individual genetic predictors can have different impact on the development of diseases in residents of different countries and regions. Therefore, regional studies are of particular interest today. As for residents of the Sumy Oblast, Ukraine, active research is being conducted on specific genetic markers of acute coronary syndrome [20, 21], ischemic stroke [22], diabetes mellitus [23], and tumors of the genitourinary system [24, 25], including benign diseases of the female reproductive system [26]. The relevance of our study is attributable to the lack of research on the role of estrogen receptor alpha gene polymorphisms in the development of EHP in the Sumy Oblast, as well as to the high frequency of EHP and the high risk of EHP transformation into a malignant process.

Currently, the pathogenesis of EHP is not fully understood. However, the role of the estrogen system and genetic factors in this process is unquestionable. It is known that ESR1 gene polymorphisms are of great importance in realizing the effects of estrogen on the functioning of a woman's body. Some of them are involved in the development of female reproductive health problems, including EHP [27]. The association of the PvuII polymorphism of the ESR1 gene with diseases of the female genital organs is still controversial. Thus, Y. Feng et al. showed in their meta-analysis that included 11 cohorts of patients that the PvuII polymorphism of the ESR1 gene was a risk factor for uterine leiomyoma [28]. M. Toprak et al., in their experimental study, which included 102 Turkish women with uterine leiomyoma, did not observe any association of the PvuII polymorphism of the ESR1 gene with the development of the disease [29]. J. Wang et al. also did not confirm the association of the PvuII polymorphism of the ESR1 gene with the development of uterine endometriosis [30].

However, many scientists demonstrated in their studies that the PvuII polymorphism of the ESR1 gene was associated with numerous diseases, such as hypertension, dyslipidemia, coronary atherosclerosis [10], type II diabetes [31, 32], sexual dysfunctions [33], lung cancer [34], coronary artery disease [35], osteoarthritis [36], osteoporosis [37], precocious puberty [38], repeated spontaneous abortions [39], chronic hepatitis B [40], systemic lupus erythematosus [41, 42], Alzheimer's disease [43], dementia [44], prostate cancer in men [45]. Genetic variation T/C of the PvuII polymorphism of the ESR1 gene may be a risk factor for hepatocellular carcinoma, gallbladder cancer [46], breast cancer [10, 47], and endometrial cancer [48]. Genotypes T/C and C/C for the PvuII polymorphism of the ESR1 gene give a poor prognosis in advanced breast cancer, but they can be considered a good predictor of the therapeutic effect of hormone therapy [49, 50]. The PvuII polymorphism of the ESR1 gene can change susceptibility to endometrial cancer [51]. There is a connection between the PvuII polymorphism of the ESR1 gene and benign dysplasia of the mammary glands, namely, the C/C homozygous state is a reliable indicator of increased proliferative activity with a tendency to atypical changes. Assessing the population of patients with benign breast dysplasia from the northeastern region of Ukraine, I. Lukavenko et al. found an association of the PvuII polymorphism with the degree of

proliferation and the level of ERα expression in breast tissue in individuals with the C/C genotype [52].

A number of studies confirmed that the C allele for the PvuII polymorphism of the ESR1 gene was a risk factor for the development of infertility [53, 54]. G. Livshyts et al. showed that the T-allele for the PvuII polymorphism of the ESR1 gene correlated with reduced ovarian reserve in Ukrainian women [55]. There is an association between the PvuII polymorphism of the ESR1 gene and the levels of C-reactive protein, testosterone, and metabolic syndrome in polycystic ovary syndrome [56].

However, in our study, no correlation was found between polymorphic variants of the ESR1 gene for the PvuII polymorphism and the development of EHP. In addition, there was no association of the studied genetic factor with the histological variant of the endometrial hyperplastic process, anthropometric parameters, age, medical history, or concomitant genital and extragenital conditions in female patients of the Sumy Oblast, Ukraine.

CONCLUSIONS / ВИСНОВКИ

There was no difference in the distribution of T/T, T/C, and C/C genotypes for the PvuII polymorphism in the estrogen receptor alpha gene between patients with endometrial hyperplastic process and the control group. There was no correlation between the genotype variant for the studied polymorphism and the histological variant of the endometrial hyperplastic process, anthropometric parameters, age, medical history, concomitant genital and extragenital conditions in patients of the Sumy Oblast, Ukraine.

PROSPECTS FOR FUTURE RESEARCH / ПЕРСПЕКТИВИ ПОДАЛЬШИХ ДОСЛІДЖЕНЬ

It is advisable to investigate the expression of estrogen receptors and genotypes for the PvuII polymorphism of the estrogen receptor alpha gene in a larger group of patients with endometrial hyperplastic processes.

CONFLICT OF INTEREST / КОНФЛІКТ ІНТЕРЕСІВ

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS / ВКЛАД АВТОРІВ

Tsyndrenko N. L.: idea and study design; data collection and analysis; statistical analysis; writing the paper; critical review; final approval of the paper.

Romanyuk A. M.: idea and study design; critical review; final approval of the paper.

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INFORMATION ABOUT THE AUTHORS / ВІДОМОСТІ ПРО АВТОРІВ

Циндренко Наталія Леонідівна, аспірант кафедри патологічної анатомії Сумського державного університету, м. Суми, Україна.
nebesenko.n@ukr.net
ORCID: https://orcid.org/0000-0001-6763-476X

Романюк Анатолій Миколайович, д.мед.н., проф., завідувач кафедри патологічної анатомії Сумського державного університету, м. Суми, Україна.
pathomorph@gmail.com
ORCID: https://orcid.org/0000-0003-2560-1382