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EPIGENETIC AND GENETIC DETERMINANTS OF ENDOMETRIAL HYPERPLASTIC PROCESSES IN PERIMENOPAUSAL OBESE WOMEN

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Abstract: Endometrial hyperplasia (EH) in perimenopause is almost always accompanied by abnormal uterine bleeding (AUB), the nature of which is due to hormonal, biochemical, molecular genetics, and local violation of the regulation of cell proliferation. The aim of the study was to search for genetic markers to increase the effectiveness of therapy in the future and reduce the frequency of relapses of EH in perimenopausal women, taking into account the metabolic profile, and the potential presence of chronic inflammation, recurrent AUB. 225 perimenopausal women were examined. The main group of women with AUB was divided into 2 subgroups: obese (n=71) and non-obese (n=49). The control group consisted of 105 healthy women. Histological examination of endometrial biopsies was performed, as well as genotyping of polymorphisms of 4 genes by PCR: s1800629I TNF-α, Arg72Pro of the TP53 gene, ER, and PR gene. The determination of a stable relationship between the identified polymorphisms of both receptor and apoptotic and pro-apoptotic genes in women with EH and obesity indicates the feasibility of conducting genetic studies to predict the risk of progression and recurrence of EH in peri - and postmenopausal women.

Keywords: perimenopause, endometrial hyperplasia, obesity, histological examination, molecular genetics study, TNF-α gene, TP53, ER and PR.

Relevance. The problem of endometrial hyperplasia (HPE) in perimenopause remains a subject of close interest due to conflicting histological, molecular-genetic, biochemical results and many markers that do not unambiguously predict the course and outcome of the disease and justify the type of therapy [3, 11]. In addition, the high recurrence rate, the lack of adequate efficacy of hormonal therapy, and the high risk of HPE malignancy dictate the need to improve the approach to its treatment [5, 23].

It is recognized that the development of HPE in perimenopause is a natural condition corresponding to hormonal homeostasis, however, HPE is diagnosed in 50-80% of women and is almost always accompanied by a clinic of abnormal uterine bleeding (AMB) [13, 14, 26, 27, 29]. It is possible to develop HPE against the background of the absence of hormonal disorders, which is associated with local dysregulation of cell proliferation and changes in tissue metabolism [3]. Against the background of hyperestrogenism, simple HPE develops, while complex and atypical HPE occurs only in the endometrial glands, focally, often against the background of atrophy and a wide range of endometrial pathology [10]. In 40-50% of cases, HPE is combined with uterine myoma [8, 14].

Obese and postmenopausal women with metabolic syndrome have a high potential for progression and recurrence of HPE due to excessive extragonadal, from

the fat depot, hyperestrogenemia. In women with normal body weight, 1% of androstenedione is converted into estrone, and in obese women, its conversion increases 10 times [9, 16]. With slight obesity, the relative risk of EC increases by about 2 times, while with a pronounced form it increases by 10 times [11, 22, 24, 28]. In addition, abdominal fat is metabolically active due to the "adipocytokines" it produces: leptin, tumor necrosis factor-α (TNF-α), etc. [15].

There is an opinion that HPE in perimenopause is a consequence of the total "accumulation" of estrogenic influences, that is, the duration of "overt" estrogenic effects is important, and not a momentary increase in the concentration of estradiol. In this connection, determining the level of hormones is not a prerequisite for identifying the cause of HPE in this period, and even more so in women with overweight and obesity [22, 28].

Traditionally, HPE has been subject to cyclic hormone therapy with progestins for the anti-relapse treatment of bleeding. In cases of the absence of bleeding, there is no reason to treat HPE as a prevention of RE and relapse, only dynamic monitoring is sufficient [1, 8, 10, 21], due to the fact that in perimenopause this the process is limited in time and does not recur in 80% of cases [10]. However, in 50% of untreated women, in 12–30% of women who received a rehabilitation course of hormone therapy for 6 months [4, 12], in 20–40% of women after discontinuation of MHT for 5 years, regardless of the type and dose [11], as well as in women with obesity, diabetes, and hypertension [13] had relapses of AUB. The occurrence of invasive EC in relapses of HPE is noted in 20-30% of cases [3, 19]. In women aged 50 years, the probability of RE is 9%, and after reaching 80 years - 60% [21].

One of the important links in the etiopathogenesis of AUB caused by HPE is chronic endometritis, in which the balance between the processes of proliferation and apoptosis is disturbed, where TNF-α plays an important role [12, 19]. With inflammatory changes in the endometrium, reception is disturbed even with unchanged hormonal ratios [11].

In modern medicine, more and more attention is paid to the search for candidate genes that are responsible for the development of endometrial hyperplastic processes and the determination of single nucleotide polymorphisms (SNPs) associated with various links of pathogenesis [25]. To date, there is an incompletely defined and ambiguous opinion about the influence of genetic factors in the development of HPE in peri- and postmenopausal women. One of the main mechanisms for the development of HPE during this period, as is known, is a noticeable hormonal imbalance. Despite this, not all peri- and postmenopausal women develop AUB that clinically confirms HPE and myometrium. The ineffectiveness of hormone therapy probably indicates the insufficiency of determining only the processes controlled by sex steroids - proliferation, hyperplasia, atrophy. Hormonal imbalance is often accompanied by inflammation of the endometrium, and therefore, it is of no small importance to determine the factors of programmed cell death - apoptosis, one of the key indicators of which is TNF, which also acts as a pro-inflammatory cytokine. Therefore, the search for other links in the pathogenesis of HPE has led to the identification of genetic factors - the determination of the polymorphism of the TNF,

ER, PR, and Tp53 genes in the development of HPE and myometrium in peri- and early postmenopause [6].

Genetic markers of polymorphic DNA variants of early diagnosis of HPE in perimenopausal women have been identified, which can be used as prognostic markers of HPE recurrence and determine the feasibility of hormonal treatment [2, 13, 17, 18, 19]. Patients homozygous for the A1A1 allele of the gene in ERa (vP-IIIa) are at risk for the development of HPE, which may be due to the adhesive properties of integrins controlled by the allelic form of the A1A1 gene in ERa. The presence of the PbAII allele in the genotype prevents the risk of developing HPE by 10 times [13]. There are data on the participation of biomolecular markers-factors in the development of HPE: in particular, the apoptosis regulator protein, prooncogene Tr-53 [12, 14, 19] and TNF-α [19]. The role of gene polymorphism and their combination in oncogenesis was confirmed by Japanese scientists: the association of the combination in the genotype of SNP309 G (GG + TG) alleles with Tp53 codon 72 Arg/Arg in the progression of HPE in EC was proved [30]. GPE occur against the background of progressive cell resistance to apoptosis, which leads to a decrease in the degree of DNA degradation, and, as a result, the proportion of proliferating cells increases. In HPE, an increase in the number of chromosome aberrations and instability of their microsatellites was revealed [20]. Changes in the genetic apparatus are interpreted by various authors ambiguously, which makes the issues of gene control over the process of endometrial proliferation not fully resolved. The study of polymorphism of the genes of the pro-inflammatory marker TNF-α, the marker of angio- and neogenesis Tp-53, as well as the genes of sex steroid receptors - ER1 and PR can provide an opportunity for early preclinical diagnosis and the search for the optimal treatment of HPE, the final stage of which may be a malignant process.

In order to optimize the management of women with HPE in the late reproductive, peri- and postmenopausal periods of life, it is necessary to search for new non-invasive methods for diagnosing and predicting relapse to prevent both the development and progression of HPE.

In perimenopause, due to high comorbidity, an individual approach is needed to solve the problem at the moment and in the future in order to avoid not only the progression and recurrence of HPE, radical gynecological operations, but also the pathological course of menopause and associated conditions [7].

Purpose of the study. Taking into account the metabolic profile, the potential presence of chronic inflammation, AUB relapses with previously prescribed hormonal therapy prompted us to search for genetic markers in order to increase the effectiveness of therapy and reduce the frequency of AUB relapses in perimenopause due to HPE.

The aim of the study was to search for genetic markers to improve the effectiveness of therapy in the future and reduce the frequency of relapses of AUB due to HPE in perimenopausal women, taking into account the metabolic profile, the potential presence of chronic inflammation, and a history of AUB relapses in anamnesis.

Material and research methods. A survey of 225 women aged 45-57 years was carried out. The main group (n=120) consisted of women with AUB. The

majority of women in perimenopause with HPE had, if not a detailed picture of the metabolic syndrome, then its key component - overweight and obesity - in 59.2%, often against the background of chronic inflammation. Taking into account the predominant risk factor for the development of AUB - obesity, the main group of women with AUB in perimenopause studied by us was divided into: A subgroup - patients with obesity, (n=71) and B subgroup - without obesity, (n=49). The control group consisted of 105 perimenopausal women without HPE and obesity.

Women with AUB underwent separate therapeutic and diagnostic curettage/aspiration of the endometrium, followed by morphological and histochemical examination of biopsy specimens, an analysis of the factors of recurrence and progression of HPE was performed, and molecular genetic markers of HPE relapses were determined for subsequent differentiated personalized treatment.

Diagnosis of overweight and obesity was carried out by calculating the body mass index (BMI) - the Quetelet index according to the Brey formula (1981):

$$BMI=(\text{weight (kg)})/〖\text{height (m)}〗^{\wedge}2 \qquad (1)$$

BMI was assessed according to the criteria of the WHO International Committee of Experts (1997): 18.5-24.9 - healthy weight; 25.0-29.9 - overweight; 30.0 and more - obesity.

Biopsies of the endometrium were obtained using an aspiration Pipel biopsy or curettage of the uterine cavity, performed under local anesthesia, as well as from the biomaterial of the removed uterus after hysterectomy.

The material was processed according to the instructions for the unification of histological and histochemical methods for examining biopsy material. The material was fixed in 10% neutral formalin (phosphate buffer pH 7.4 for 24 h at room temperature). After washing in running water, they were dehydrated in alcohols of increasing concentration and chloroform, then embedded in paraffin with wax. Serial sections were prepared from the prepared paraffin blocks, which were obtained on an MS-2 microtome with a thickness of 3-5 μm.

For histological examination, the sections were deparaffinized, stained with hematoxylin and eosin. The preparations were studied under an MBI-6 (Russia) and Leica (Germany) light-optical microscope, micrometer-15 (OM) eyepiece.

The stages of molecular genetic research were: blood sampling and isolation of genomic DNA from lymphocytes; detection of polymorphic loci (PCR analysis); separation of amplified fragments by electrophoresis and visualization of the results.

For PCR, blood samples were taken into EDTA tubes by Vacutainer Becton Dickinson International (USA). Genomic DNA was isolated from peripheral blood lymphocytes by standard phenol-chloroform deproteinization (Sambrook J., Fritsh E. F., Maniatis T., 1989) with some modifications using the Ribo-prep reagent kit, InterLabService LLC (Russia, Moscow), Quality DNA samples were tested on a NanoDrop 2000 Thermo Scientific spectrophotometer (USA). We performed genotyping of polymorphisms localized in the promoter regions of 4 genes: rs1800629I TNF-α in positions, G-308A, of the gene, Arg72Pro of the TP53 gene, as well as G/A (rs2228480) of the ER gene and G/T (rs1042838) of the PR gene with a kit for typing by NPF Litekh LLC and NPO Sntol (Moscow).

Amplification of the studied rs1800629I TNF-α loci at positions G-308A of the Arg72Pro gene of the TP53 gene, as well as G/A (rs2228480) of the ER gene and G/T (rs1042838) of the PR gene was performed using a GeneAmp PCR-system 2720 thermal cycler (Applied Biosystems, USA) and CG1-96 (Corbett Research, QUAGEN Germany) by allele-specific PCR and real-time PCR.

Amplification for polymorphisms of the above genes was carried out under the following conditions: preliminary denaturation - 95°C (5 min), 40 amplification cycles: 95°C (1 min) - denaturation, 59°C (1 min) - primer annealing, 72°C (1 min) - elongation, and final synthesis 72°C (3 min). The pUC19 plasmid cleaved with the MspI restriction enzyme (SibEnzyme, Novosibirsk) was used as a DNA fragment length marker.

After the end of the allele-specific PCR, the specificity of the amplification and the amount of the resulting amplification were checked by electrophoresis (in 2% agarose gel). PCR products were separated by horizontal electrophoresis in 2% agarose gel containing ethidium bromide (EtBr). A 10 μl DNA sample was mixed with 2 μl of a standard solution of bromophenol blue (10%). A standard marker (Lader) was applied to the agarose gel to determine the size of the DNA bands and a control sample represented by a master mix without DNA. After electrophoresis, the gel was interpreted using a UV transilluminator with a built-in camera.

Statistical processing of the obtained data was performed on a computer using Microsoft Excel 7.1 spreadsheets and the Statistika software package, version 7, StatSoft Inc. (USA).

Statistical criteria were parametric - Student's t-test and non-parametric - Wilcoxon's signed-rank t-test and Mann-Whitney U-test for significance level $P \leq 0.05$. The significance of differences in frequencies in the groups was assessed using the $\chi 2$ test and Fisher's exact test, the differences were also considered significant at $P<0.05$. Data under the condition of normal distribution are presented as the arithmetic mean of the studied indicator (M), standard deviation (□), standard error of the mean (m), relative values (frequency, %).

Estimated deviation of the genotype distributions of the studied DNA loci (rs1800629I TNF-α at positions G-308A of the gene, Arg72Pro of the TP53 gene, as well as G/A (rs2228480) of the ER gene and G/T (rs1042838) of the PR gene) from the canonical Hardy-Weinberg distribution (RCM) was carried out using the program "GenePop" ("Genetics of Population"), (http://wbiomed.curtin.edu.au/genepop).

Allele and genotype frequencies (f) of the studied genes were calculated using the standard formula:

$$f=n/2N \text{ и } f=n/N \quad (2),$$

where n is the number of times the allele occurs,

N - the number of examined.

The coefficient of deviations of the actual heterozygosity of the studied loci from the theoretical one was calculated by the formula:

$$F=(Hexp - Hobs)/Hexp. \quad (3)$$

where Hexp and Hobs are the expected and observed frequencies, respectively.

The predictive value of each genetic marker was determined using a program available on the Internet (http://vigg.ru/fileadmin/user_upload/Rubanovich): if AUC<0.5, then the marker is random; 0.5<AUC>0.6 - poor, 0.6<AUC>0.7 - average; 0.7<AUC>0.8 - good; AUC>0.8 is an excellent classifier.

The software package "OpenEpi 2009, Version 2.3" was used as a calculation tool for molecular genetic studies. Results processing model: Case-control.

The degree of associations was assessed in terms of the odds ratio Odds ratio (OR) and the risk of developing Relative risk (RR) and their 95% confidence interval (95% CI). The value OR=1 (RR=1) showed no association. The value OR>1(RR>1) was considered as a positive association of the disease with the symptom (increased risk factor), OR<1 (RR<1) as a negative association (low risk factor).

Results of the study and their discussion. In per menopause with normal body weight, AUB (B subgroup) were 3 times more likely to be due to secretory transformation of the endometrium (10.2%), which is considered as a variant of the norm, as well as atrophy (10.2%) than in obese women (Table 1).

Obesity (A subgroup) was accompanied by the following histological nature of AUB in perimenopause: complex HPE in 12 (16.9%, OR=1.5; RR=1.4), including complex HPE with atypia in 3 (4.2%) and transformation into a malignant process - in 2 (2.8%), as well as more frequent organic - fibroids - in 31 (43.7%, OR=3.4; RR=2.4) and the combined nature of bleeding due to combined histological picture - in 63 (88.7% OR=5.9; RR=1.6) versus 57.1% in non-obese women (P<0.05).

Consequently, obesity exacerbates the nature of hyperplastic processes in the endo- and myometrium, accompanied by AUB in perimenopause and early postmenopause, and contributes to the progression up to atypization and malignancy, while in women with normal body weight, secretory processes are more often observed (3.6 times, OR=0, 3; RR=0.3) and atrophic (2.4 times, OR=0.4; RR=0.4) changes in the endometrium with inflammation (1.2 times, OR=0.8; RR=0 ,eight).

A molecular genetic study of the rs2228480/594 polymorphism of the ER gene showed that the frequency of G/G, G/A, A/A genotypes in the main group was 81.7; 15.8 and 2.5, and in the control - 81.9; 16.2 and 1.9% (Table 2). No significant and statistically significant differences in gene polymorphism were found. Therefore, both in the main and in the control groups, the expression of the normal ER genotype (G/G) was most often observed. Therefore, the response to estrogen therapy will be adequate and HPE is due to hyperestrogenemia.

Table 1.

Results of histomorphological examination of the endometrium in women with AUB, abs, %

Histomorphological picture	Sub-group A, n=71		Sub-group B, n=49		OR	RR
	абс	%	абс	%		
GGE, of which:	53	74,6±5,2	35	71,4±6,5	1,2	1,0
Simple GGE	41	57,7±5,9	29	59,2±7,0	0,9	1,0
- Simple HPE without atypia	27	38,0±5,8	17	34,7±6,8	1,2	1,1
Mixed endometrium	7	9,9±3,5	3	6,1±3,4	1,7	1,6

Secretory transformation	2	2,8±2,0	5	10,2±4,3	0,3	0,3
Dystrophic changes	4	5,6±2,7	4	8,2±3,9	0,7	0,7
- Simple HPE with atypia	1	1,4±1,4	-	-		
Complex GGE	12	16,9±4,4	6	12,2±4,7	1,5	1,4
- Complicated without atypia	9	12,7±3,9	6	12,2±4,7	1,0	1,0
- Complicated with atypia	3	4,2±2,4	-	-		
Adenocarcinoma	2	2,8±2,0	-	-		
endometrial cancer	-	-	1	2,0±2,0		
Polyp / polypoid fragments of the endometrium	8	11,3±3,8	4	8,2±3,9	1,4	1,4
Scanty scraping, small scraps of endometrium.	3	4,2±2,4	4	8,2±3,9	0,5	0,5
Atrophy or transitional type of endometrium	3	4,2±2,4	5	10,2±4,3	0,4	0,4
Uterine fibroids	31	43,7±5,9	9	18,4±5,9*	3,4	2,4
endometritis	18	25,4±5,2	15	30,6±6,6	0,8	0,8
Combined histopicture	63	88,7±3,8	28	57,1±7,1*	5,9	1,6

* P>0.05 differences are significant

Table 2.

The frequency of distribution of alleles and genotypes of the G/A polymorphism of the ER gene (rs2228480) in groups of patients with AUB and controls

№	Group		Allele frequency				Frequency distribution of genotypes					
			G		A		G/G		G/A		A/A	
			n	%	n	%	n	%	n	%	n	%
1	Main Group AMK	120	215	89,6	25	10,4	98	81,7	19	15,8	3	2,5
А	AMK with obesity	71	121	85,2	21	14,8	53	74,6	15	21,1	3	4,2
Б	BUN without obesity	49	94	95,9	4	4,1	45	91,8	4	8,2	0	0
2	Control group	105	189	90,0	21	10,0	86	81,9	17	16,2	2	1.9

When comparing the results of a genetic study in women with AUB against the background of obesity (A subgroup) with the control group, a statistically insignificant difference was found in the frequency (2.2 times more often) of the occurrence of the homozygous rare A/A genotype ($\chi 2<0.8$; P= 0.3; RR=2.2; 95% CI 0.380-12.94; OR=2.3; 95% CI 0.369-13.95). At the same time, when comparing the genotypes of patients of the main subgroups A and B, it was found that the frequency of the heterozygous genotype (G / A) in women with AUB against the background of

obesity increases by 2.6 times than in women with AUB without obesity ($\chi 2$ = 3 .7; P=0.06; RR=2.6; 95% CI 0.913-7.33; OR=3.0; 95% CI 0.9347-9.715), as well as the absolute predominance of the rare homozygous ER gene - A /A in 4.2% of cases.

At the same time, the G/G genotype played a protective role in the development of HPE only against the background of obesity ($\chi 2$=5.7; P=0.02; RR=0.8; 95% CI 0.6932-0.9531; OR=0, 3; 95% CI 0.0825-0.8298), which can be explained by the low level of ER gene transcription in HPE. Therefore, the determining factor is the presence of a background of obesity in women with AUB, in which the G/G genotype (rs2228480) of the ER gene is less frequently observed.

The study of allelic and genotypic variants of the polymorphism (promoter region) G/T (s1042838) of the PR gene did not show a significant difference in the occurrence of G alleles in the main (85.4%) and control (91%) groups ($\chi 2$=3.2; P=0 .07; RR=1.6; 95% CI 0.951-2.73; OR=1.7; 95% CI 0.949-3.103), and the T allele in 14.5 and 9%, respectively (Table 3) . But a significant difference (5.8 times more often) was determined in the frequency of detection of the homozygous rare T/T genotype in patients with AUB compared with the control group ($\chi 2$=3.9; P=0.04; RR=6.1; 95% CI 0.7662-48.97; OR=6.4; 95% CI 0.7795-53.25).

Table 3

The frequency of distribution of alleles and genotypes of the G/T (rs1042838) polymorphism of the PR gene in groups of patients with AUB and controls

№	Group		Allele frequency				Frequency distribution of genotypes					
		n	G		T		G/G		G/T		T/T	
			n	%	n	%	n	%	n	%	n	%
1	Main Group AMK	120	205	85,4	35	14.6	92	76,7	21	17,5	7	5,8
А	AMK with obesity	71	117	82,4	25	17.6	52	73,2	13	18,3	6	8,4
Б	BUN without obesity	49	88	89,8	10	10,2	40	81,6	8	16,3	1	2,0
2	Control group	105	191	91	19	9,0	87	82,9	17	16,2	1	1,0

This may indicate a lack of response to traditional progesterone anti-relapse therapy in AUB. Since it was women with the T/T genotype who had recurrent bleeding during hormone therapy, and most of them were obese and GB. Thus, when comparing genotypic polymorphism, as in the previous comparison, a rare homozygous T/T gene was detected more often in women with recurrent AUB against the background of obesity ($\chi 2$=6.2; P=0.01; RR=8.9; 95 %CI 1.09-72.12; OR=9.6; 95%CI 1.13-81.55).

At the same time, comparison of the polymorphism results of women with AUB without obesity with the control group showed no differences, therefore, it follows that obesity is a fundamental factor in the development of AUB in perimenopause and it is encoded by a homozygous rare carriage of the T/T promoter of the PR gene. This evidence was provided by the results of comparison of the main subgroups - thus, the odds (OR) and risk (RR) of developing AUB in women with obesity and the frequency of the T/T genotype are 4.1 and 4.4, respectively ($\chi 2$=2.2; P=0 .1; RR=4.1; 95% CI 0.5146-33.32; OR=4.4; 95% CI 0.5164-38.02). The calculations showed the prognostic efficiency of the G/T polymorphism (rs1042838) of the PR gene in women with AUB against the background of obesity (OR=2.1; 95% CI 1.13-4.07; P=0.02).

As is known, TNF-α is a pro-inflammatory cytokine involved in the processes of apoptosis, cell differentiation and proliferation. The study of its polymorphism in women with HPE is of great interest because, on the one hand, it is "universal" in terms of inflammation and, on the other hand, its influence on the processes of progression of growth/proliferation. Thus, we found a relationship between a more frequent (1.6 times) heterozygous G/A state of the rs1800629 TNF-α gene and the development of AUB in perimenopausal women, both with obesity (29.6%) and without obesity (30, 6%) compared with healthy women (19.1%) (Table 4).

So, it turned out to be statistically significant in the main group as the prevalence of allele A ($\chi 2$=4.2; P=0.04; RR=1.7; 95% CI 1.012-2.87; OR=1.8; 95% CI 1.018 -3.297) and G/A genotype ($\chi 2$=4.3; P=0.04; RR=1.7; CI 1.016-2.707; OR=1.9; 95% CI 1.031-3.649) over control. It should be noted that 33 (27.5%) patients with HPE also had a histological picture of chronic endometritis. When analyzing the results of a molecular genetic study, it was found that 2/3 of women with HPE against the background of chronic inflammation of the endometrium (25 out of 33 - 75.8%) are carriers of G/A. In view of the results obtained, it is possible to present the definition of TNF-α gene polymorphism as a prognostic marker for the development of HPE and endometrial hyperplasia against the background of chronic endometritis (OR=1.8; 95% CI 1.018-3.297; P=0.04).

Table 4

The frequency of distribution of alleles and genotypes of the rs1800629 polymorphism of the TNF-α gene in groups of patients with AUB and controls

№	Group		Allele frequency				Frequency distribution of genotypes					
			G		A		G/G		G/A		A/A	
			n	%	n	%	n	%	n	%	n	%
1	Main Group AMK	120	203	84.6	37	15,4	84	70,0	36	30,0	0	0
А	AMK with obesity	71	121	85.2	21	14,8	50	70,4	21	29,6	0	0
Б	BUN without	49	83	84,7	15	15,3	34	69,4	15	30,6	0	0

	obesity											
2	Control group	105	191	90,9	19	9,0	86	81,9	19	19,1	0	0

Another key protein regulating cell proliferative activity is the proapoptotic Tr53 protein. The study of the gene polymorphism of this marker is relevant due to its role in carcinogenesis. The processes of proliferation of endo- and myometrium today are considered as a single process of growth from benign to malignant. The distribution frequency of alleles and genotypes of the Arg72Pro polymorphism in the TP53 gene in the group of women with AUB showed a 1.5-fold prevalence of the Pro allele frequency compared to the control group ($\chi2$=6.7; P=0.01; RR=1.5; 95 %CI 1.102-2.114; OR=1.8; 95%CI 1.146-2.72), while homozygous carriage of Arg/Arg can be considered as a relative protector of the development of HPE in perimenopause ($\chi2$=6.3; P=0.01 ; RR=0.7; 95% CI 0.5628-0.933; OR=0.5; 95% CI 0.2976-0.8631) (Table 5).

Table 5

Distribution frequency of alleles and genotypes of Arg72Pro polymorphism in the TP53 gene in groups of patients with AUB and controls

№	Group		Allele frequency				Frequency distribution of genotypes					
			Agr		Pro		Arg/Arg		Arg /Pro		Pro/Rro	
			n	%	n	%	n	%	n	%	n	%
1	Main Group AMK	120	165	68,8	75	31,2	53	44,2	59	49,2	8	6,7
А	AMK with obesity	71	90	63,4	52	36,6	25	35,2	40	56,3	6	8,4
Б	BUN without obesity	49	75	76,5	23	23,5	28	57,1	19	38,8	2	4,1
2	Control group	105	167	79,5	43	20,5	64	60,9	39	37,1	2	1,9

The rare homozygous Pro/Pro genotype was more common in women with AUB, and more pronounced and significant against the background of obesity ($\chi2$=4.2; P=0.04; RR=4.4; OR=4.7), then how Arg/Arg acted as a protector of the development of AUB against the background of obesity ($\chi2$=11.2; P<0.05; RR=0.6; 95%CI 0.4068-0.8203; OR=0.3; 95 % CI 0.1863-0.6506) and was observed 1.73 times less frequently than in the control group and 1.82 times less frequently than in the non-obese AUB group ($\chi2$=5.6; P=0.02 ; RR=0.6; 95% CI 0.4139-0.9173; OR=0.4; 95% CI 0.1932-0.8599).

Therefore, Arg/Arg can be considered as a protector of the development of AUB against the background of obesity, as well as Pro/Pro as its predictor. Prognostic

efficiency of the Arg72Pro polymorphism in the TP53 gene in women with AUB and obesity (OR=2.2; 95% CI 1.391-3.621; P<0.05) served as proof of this.

Thus, the genetic determinants of HPE are the detection of the rs2228480/594 polymorphism of the ER gene. The G/G genotype plays a protective role in the development of HPE only against the background of obesity ($\chi 2$=5.7; P=0.02; RR=0.8; 95%CI Dominance of a rare homozygous variant of T/T polymorphism (s1042838) of the PR gene in women with recurrent AUB against the background of obesity ($\chi 2$=6.2; P=0.01; RR=8.9; 95% CI 1.09-72.12 ; OR = 9.6; 95% CI 1.13-81.55) may reliably indicate a lack of response to traditional progesterone anti-relapse therapy in AUB.

In women with AUB, both with and without obesity, there is a statistically significant prevalence of both the A allele ($\chi 2$=4.2; P=0.06; RR=1.7; OR=1.8; 95% CI 1.02 -3.29) and the G/A rs1800629 genotype of the TNF-α gene ($\chi 2$=4.3; P=0.04; RR=1.7; OR=1.9; 95% CI 1.03-3 ,65) over the control group.0.6932-0.9531; OR=0.3; 95%CI 0.0825-0.8298).

We revealed the prognostic efficiency of the Arg72Pro polymorphism marker in the TP53 gene in women with AUB and obesity (OR=2.2; 95% CI 1.391-3.621; P<0.05). The Arg/Arg genotype can be considered a reliable protector of the development of AUB against the background of obesity, while Pro/Rro is its predictor.

Determination of a stable relationship between the identified polymorphisms of both receptor and apoptotic and proapoptotic genes in women with HPE and obesity indicates the feasibility of genetic studies, especially with a burdened family history of cancer, to predict the risk of progression and recurrence of HPE in peri- and postmenopause. The choice of tactics for further treatment depended on the histological result, the phase of the woman's life, the presence of somatic pathology (including obesity), the presence and severity of the climacteric syndrome, which is more often caused by an important factor in the duration of compliance - the informed choice of the patient herself. Undoubtedly, the anamnesis, the results of genetic testing, the presence of contraindications to hormone therapy, and the absence of indications for surgical treatment were also taken into account. The management program for women with AUB due to HPE in peri- and postmenopause should be formed not only taking into account the possible hormonal and metabolic background, the data of a burdened somatic and gynecological, family history, but also taking into account the results of a genetic study. This will enable a differentiated, purposeful, or rather personalized approach to the appointment of a therapeutic and prophylactic approach, including the appointment of MHT, the possibility of continuing to take it or canceling it. Conducting a comprehensive examination, including genetic research, is a fairly promising direction in the prevention and early diagnosis of HPE in women with risk factors, especially those prone to malignancy.

Conclusion. We have determined a significant relationship between the formation and development of HPE on the background of obesity with the carriage of an unfavorable variant of the T/T polymorphism of the PR gene (RR=8.9; OR=9.6); HPE against the background of inflammation - with the G/A rs1800629

polymorphism variant of the TNF-α gene (RR=1.5; OR=1.8); endometrial hyperplasia - with the Pro Arg72Pro allele of the Tp53 gene (RR=1.5; OR=1.8), and the Arg/Arg variant is significantly associated with a reduced risk of endometrial hyperplasia (protective effect) (RR=0.7; OR=0 ,5).

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