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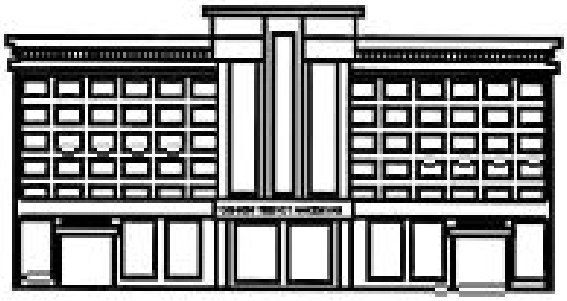

ВЕСТНИК

ТАШКЕНТСКОЙ МЕДИЦИНСКОЙ АКАДЕМИИ

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POLYMORPHISM OF THE ESTROGEN RECEPTOR GENE IN THE IN WOMEN WITH UTERINE FIBROIDS

Yuldasheva D.Y., Irnazarova D.Kh., Kayumova D.T., Irnazarov A.A., Parvizi N.I.

ПОЛИМОРФИЗМ ГЕНА РЕЦЕПТОРА ЭСТРОГЕНА У ЖЕНЩИН С МИОМОЙ МАТКИ

Юлдашева Д.Ю., Ирназарова Д.Х., Каюмова Д.Т., Ирназаров А.А., Парвизи Н.И.

BACHADON MIOMASI BO'LGAN AYOLLARDA ESTROGEN RESEPTORLARI GENINING POLİMORFIZMASI

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Миома матки является одной из наиболее частых доброкачественных опухолей у женщин в структуре гинекологических заболеваний. Несмотря на свою распространенность, миома остается относительно малоизученной нозологией. Нами обследовано 200 женщин репродуктивного и пременопаузального возраста в Многопрофильной клинике Ташкентской медицинской академии с 2018-2020 гг. Женщины были разделены на группы: основная группа (n=102) - с симптомной (n=53) и бессимптомной (n=49) миомой и контрольная группа (n=98). Всем женщинам были проведены общеклинические и молекулярно-генетические исследования. Полиморфный маркер rs2228480/594 гена ESRa была ассоциирован с развитием миомы матки у обследованных женщин.

***Ключевые слова:** миома матки, полиморфизм, ген рецептора ESRa.*

Bachadon miomasi ginekologik kasalliklar tarkibida ayollarda eng ko'p uchraydigan yaxshi xulqli o'smalardan biridir. Keng tarqalganligiga qaramay, mioma nisbatan kam o'rganilgan nozologiya bo'lib qolmoqda. 2018-2020-yillarda Toshkent tibbiyot akademiyasi ko'p tarmoqli klinikasida reproduktiv va premenopozal yoshdagi 200 nafar ayolni tibbiy ko'rikdan o'tkazdik. Ayollar guruhlarga bo'lingan: asosiy guruh (n=102) - simptomatik (n=53) va asemptomatik (n=49) mioma bilan va nazorat guruhi (n=98). Barcha ayollar umumiy klinik va molekulyar genetik testlardan o'tkazildi. ESRa genining rs2228480/594 polimorf markeri tekshirilgan ayollarda bachadon miomasining rivojlanishi bilan bog'liq edi.

***Kalit so'zlar:** bachadon miomasi, polimorfizm, ESRa retseptorlari geni.*

Клиническая медицина

Relevance. Uterine fibroids (UF) are one of the most common benign tumors in women, occupying one of the first places in the structure of gynecological diseases (SOCG, 2015). The prevalence of UF ranges from 12-25 to 70-80% of the total number of gynecological diseases, reaching a maximum in late reproductive and premenopausal age [7,10]. Traditionally, one of the main inducers of fibroids growth through their receptors are estrogens, thanks to which conditions for progesterone-mediated growth are formed in target tissues [3,8]. Currently, the association of the polymorphic locus of the estrogen receptor gene (ERa) involved in the pathogenesis of fibroids, which is localized on chromosome 6q25, is actively studied worldwide [5,12]. UF is a disease of a multifactorial nature, which is based on the combined effect of genetic and epigenetic factors and there is an urgent need to continue studying the etiopathogenesis of fibroids.

The aim of the study was to study the relationship of polymorphic variant of estrogen receptor genes ERa (rs2228480/594) with the development of uterine fibroids.

Materials and methods of research. An open prospective cohort study was conducted in the period from 2019 to 2022, which was based on a clinical and laboratory examination of 200 women of reproductive and premenopausal age who were admitted to the Women's Health Center and the Department of Gynecology of the Multidisciplinary Clinic of the Tashkent Medical Academy. The women were divided into groups: the main (n=102) patients with UF, which in turn was divided into 2: with symptomatic (n=53) and asymptomatic (n=49) UF and the control group (n=98), which consisted of healthy women.

When working with women, the ethical principles presented by the Helsinki Declaration of the World Medical Association "Ethical Principles of scientific and medical research with human participation" (revised in 2013) [11] and diagnostic measures were observed, according to the National Protocol of the Ministry of Health of the Republic of Uzbekistan [9]. All women were conducted: collection of complaints and anamnesis, general and gynecological examinations, routine clinical and laboratory studies, anthropometry, calculation of body mass index (BMI).

Inclusion criteria: patients diagnosed with uterine fibroids with symptomatic and asymptomatic course and age from 18 to 54 years, conditionally healthy women without UF of comparable age, menstruation, informed consent of the woman for examination.

Exclusion criteria: the age of women under 18 and over 54 years, pregnant women, patients registered at the dispensary; alcohol abuse, drug use, the presence of malignant neoplasms, chronic diseases in the stage of decompensation or exacerbation, the refusal of a woman to participate in the study.

Genetic studies were conducted at the Research Institute of Hematology and Blood Transfusion, in the Department of Molecular Medicine and Cellular Technologies. The stages of the study were: blood sam-

pling and isolation of genomic DNA from lymphocytes; detection of polymorphic loci (PCR analysis); separation of amplified fragments using electrophoresis and visualization of the results. For PCR, blood samples were collected in test tubes with EDTA 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 a set of reagents "Rib-prep" LLC "InterLabService" (Russia, Moscow). The quality of DNA samples was tested on a NanoDrop 2000 "Thermo Scientific" spectrophotometer (USA). Genotyping of polymorphisms localized in promoter regions of the G/A receptor genes of the ESRa gene (rs2228480/594) was carried out with a typing kit from the company NPF Litech LLC and NPO Sntol (Moscow). Amplification of the studied G/A loci of the ESRa gene (rs2228480/594) was carried out using GeneAmp PCR-system 2720 thermal cyclers (Applied Biosystems, USA) and CG1-96 (Corbett Research QUAGEN Germany) by allele-specific PCR and real-time PCR.

Results and discussion. The analysis of the age characteristics of the women studied showed that the average age was 18-54 years. The highest average age is observed in women of the main group: with asymptomatic myoma - 42.6±1 years (n=49) and with symptomatic - 43.5±0.2 years (n=53), whereas in the control group (n=98) the average age was 38.7± 0.9 years (p<0.001).

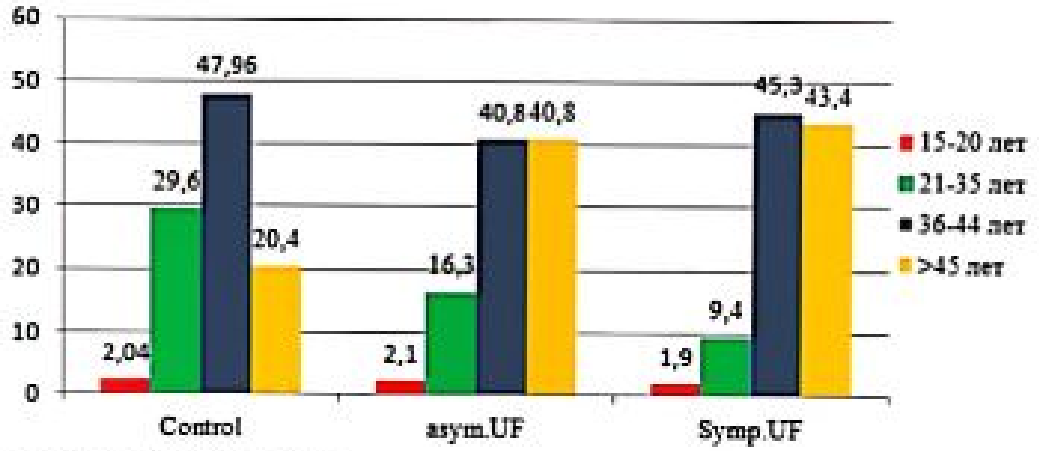

Fig. 1. Age of the examined patients, %.

When analyzing risk factors for UF, we found that the risk of developing fibroids are (symptomatic and asymptomatic fibroids, respectively): age (OR=1.8 and 2.1), burdened heredity (OR=3.1 and 3.0), previously menarche (OR=5 and 1.3), obesity (OR=6.4 and 5.7), insufficient insolation (OR=53.1 and 3.0), the use of injectable progestogens (OR=14.3 and 3.7) and COCs (OR=3.15), curettage of the uterine cavity (OR=3.75 and 2.8), inactivity (OR=5.1 and 1.5), closed clothing (OR=5 and 2.4), stress (OR=5 and 1.75) [4,5].

Women with symptomatic UF of the main group (n=53) treated with various clinical manifestations: the symptom of bleeding and anemia prevailed to a greater extent in 86.8% (n=46), of which 18.9% of women underwent hemotransfusion due to severe anemia; the symptom of rapid growth – 11.3% (n=6), the symptom of pelvic pain (n=5) 9.4% and infertility symptom in 7.5% (n=4).

The results of surgical treatment of women with symptomatic UF (n=53) were analyzed. The criteria for choosing an operative method of UF treatment were age, the presence of reproductive goals, and the severity of clinical symptoms in the studied women with symptomatic fibroids.

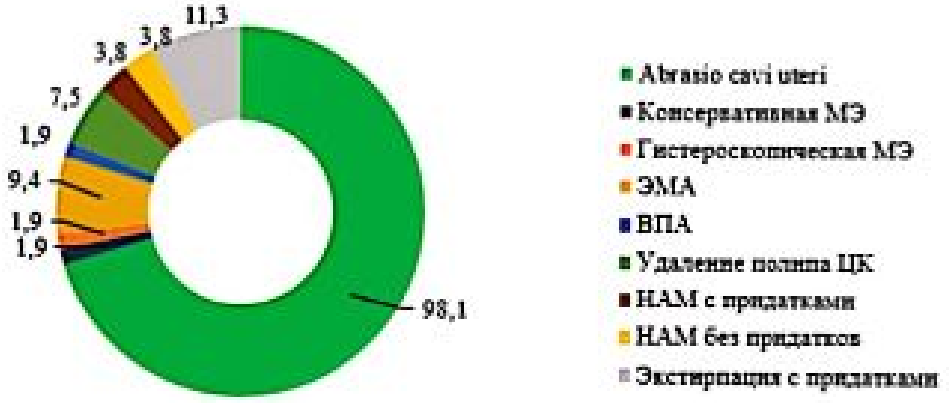

Fig.2. Types of surgical interventions in women with symptomatic UF, n=53,%

All women in the main group were clinically diagnosed with uterine fibroids by ultrasound, which revealed the number and localization of myomatous nodes. We have analyzed the relationship between the features of the number and location of myomatous nodes with the presence and absence of a myoma clinic. In women with symptomatic UF (n=53), 1/3 (32.1%) of women had multiple UF (more than 2 myomatous nodes) and 2/3 (67.9%) had a solid tumor, whereas in the subjects with asymptomatic UF, the multi-node UF was found to be 2 times less than the symptomatic UF (14.3% and 85.7% respectively). The number of myomatous nodes in the studied women of the main group with multiple nodes varied from 2 to 6 nodes.

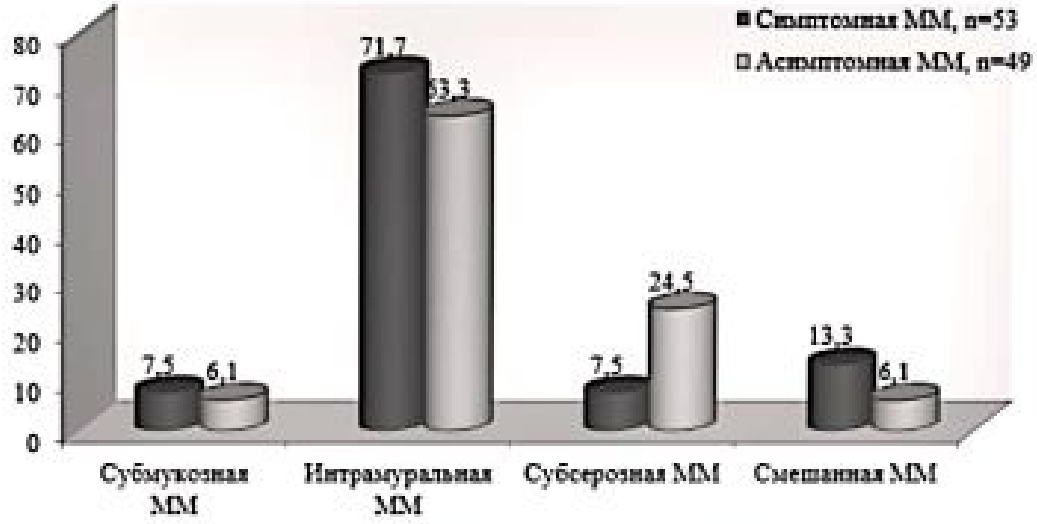

Fig.3. The location of myomatous nodes in the thickness of the uterus in women of the main group (n=102), abs %

According to the localization of the myomatous node in the thickness of the uterus in both groups of the main group, the intramural node prevailed (71.7% and 63.2%, respectively, groups), submucous (7.15% and 6.12%) nodes in an equal ratio and subserous (7.15% and 24.5%) were detected 3 times more often in women with asymptomatic UF. Mixed myomatous nodes in women with symptomatic MM were 2 times more common than in women with asymptomatic UF (13.2% and 6.12%, respectively). The median uterine volume during ultrasound examination (Brunn, 1981) in the group with symptomatic UF was 237.54 cm3, asymptomatic UF - 103.45 cm3 and in the control group - 52.1 cm3 ($p < 0.01$).

In order to optimize the diagnosis of women with UF in the reproductive and perimenopausal periods of life, the search for new non-invasive methods of diagnosis and prognosis of the clinical course and complications of the disease, to prevent both the development and progression of the disease, leading to radical management tactics, prompted us to conduct molecular genetic studies and study the associative roles of genetic polymorphism of the ESRa receptor gene. (rs2228480/594) with this pathology. The distribution of genotypes in the control and main groups according to the studied genes corresponds to the Hardy-Weinberg equilibrium (RHB).

Table 1

Frequency of distribution of alleles and genotypes of the rs2228480/594 polymorphism of the ESRa gene in the main group with UF and control group

№	Groups	n	Frequency of distribution of alleles				Frequency of distribution of genotypes					
			G		A		G/G		G/A		A/A	
			n	%	n	%	n	%	n	%	N	%
1	main group	102	173	84.8	31	15.2	74	72.5	23	22.5	5	4.9
a	Symp. UF	53	90	84.9	16	15.1	38	71.7	14	26.4	1	1.9
b	Asymp. UF	49	81	82.7	17	17.3	36	73.5	9	18.4	4	8.2
2	control	98	178	90.8	18	9.2	82	83.7	14	14.3	2	2.0

As a result of the analysis of the frequency of distribution of alleles and genotypes of the rs2228480/594 polymorphism of the ESRa gene in the patient and control groups, we found that the frequency of the G allele in the control and main groups is 90.8% and 84.8%, respectively. Unfavorable allele A in the sample was 1.6 times less common (9.2%) compared to the main group (15.2%). The predominant genotype in the study was a favorable G/G genotype in the control group it was 83.7% and in the main group – 72.5%. Nevertheless, the frequency of homozygous mutant genotype A/A in the group with asymptomatic UF is 4.1 times higher than the control indicators – 8.2% vs. 2.0%, and among women with symptomatic UF - 4.3 times (8.2% vs. 1.9%), as a result, in the main group, the subjects with homozygous genotype AA are 4.9% (Table 1).

Table 2

Differences in the frequency of occurrence of alleles and genotypes of the rs2228480/594 ESRa gene polymorphism in women with symptomatic UF and control

Alleles/ genotypes	Symp UF, n=53		Control n=98		χ2	P	RR	95% CI	OR	95% CI
	n	%	n	%						
G	90	84.9	178	90.8	2.4	0.1	1.6	0.874- 3.088	1.8	0.856- 3.61
A	16	15.1	18	9.2						
G/G	38	71.7	82	83.7	3.0	0.08	0.9	0.708-1.037	0.5	0.221-1.103
G/A	14	26.4	14	14.3	3.3	0.07	1.8	0.954-3.581	2.1	0.9360-4.95
A/A	1	1.9	2	2.0	0.004	0.9	0.9	0.085- 9.959	0.9	0.081- 10.42

When analyzing the differences in the frequency of occurrence of alleles and genotypes of the rs2228480/594 polymorphism of the ESRa gene in the group of women with symptomatic UF and the control sample, it was found that the risk of developing UF with heterogeneous unfavorable genotype G/A was 2.1 times significantly higher compared to the control group (χ^2=3.3; P=0.07; RR=1.8; 95%CI 0.954-3.581; OR=2.1; 95% CI 0.9360-4.95). At the same time, the G/G genotype played a protective rule in the development of UF (Table 2).

The rs2228480/594 polymorphism of the ESRa gene in the group of women with asymptomatic UF and the control sample showed that the protective genotype G/G was more common in the control group (OR=0.5; 95% CI 0.235-1.238).

Table 3

Differences in the frequency of occurrence of alleles and genotypes of the rs2228480/594 ESRa gene polymorphism in women with asymptomatic UF and control

Alleles and genotypes	Asymp UF, n=49		Control, n=98		χ2	P	RR	95% CI	OR	95% CI
	n	%	n	%						
G	81	82.7	178	90.8	4.1	0.04	1.9	1.019-3.5	2.1	1.017-4.234
A	17	17.3	18	9.2						
G/G	36	73.5	82	83.7	2.1	0.1	0.9	0.726- 1.061	0.5	0.235- 1.239
G/A	9	18.4	14	14.3	0.4	0.5	1.3	0.598- 2.76	1.3	0.539- 3.381
A/A	4	8.2	2	2.0	3.1	0.08	4.0	0.758- 21.09	4.3	0.7535- 24.16

Nevertheless, when comparing homozygous unfavorable genotypes, A/A in women with asymptomatic UF increases 4.3 times higher than in the control sample (χ2=3.1; P=0.08; RR=4; 95% CI0.758-21.09; OR=4.3; 95% CI 0.753-24.16) (Table.3).

Table 4

Differences in the frequency of occurrence of alleles and genotypes of the rs2228480/594 ESRa gene polymorphism in the groups of symptomatic and asymptomatic UF

Alleles and genotypes	Asymp UF, n=53		Symp UF, n=49		χ2	P	RR	95% CI	OR	95% CI
	n	%	n	%						
G	90	84.9	81	82.7	0.2	0.7	1.1	0.615- 2.147	1.2	0.56- 2.489
A	16	15.1	17	17.3						
G/G	38	71.7	36	73.5	0.04	0.8	1.0	0.807- 1.301	1.1	0.457-2.61
G/A	14	26.4	9	18.4	0.9	0.3	0.7	0.33-1.46	0.6	0.243- 1.615
A/A	1	1.9	4	8.2	2.1	0.1	4.3	0.501- 37.38	4.6	0.49-42.87

When comparing the genotypes of patients with symptomatic and asymptomatic UF, it was revealed that the frequency of mutant homozygous genotype A/A in women with asymptomatic UF is 4.6 times more common than in women with symptomatic UF (χ^2=2.1; P=0.1; RR=4.3; 95%CI 0.501-37.38; OR=4.6 95% CI 0.49-42.87), as well as the absolute predominance of the rare homozygous ESRa - A/A gene in 8.2% of cases (Table 4). This explains that women with symptomatic UF with multiple epigenetic factors for the disease develop a vivid clinical picture, whereas women with asymptomatic UF with low risk factors, even with more frequent mutant genes, do not clinically manifest themselves. These differences in the frequency of occurrence of the wild genotype in women with UF can he explained according to the authors' data that ESRa polymorphism affects the development of fibroids mediocre [1, 2, 6].

To study the prognostic significance of the studied receptor gene polymorphism for predicting UF risk, Se, Sp and the prognostic efficacy of the marker AUC were determined as separate genetic predictors (Table 5).

Table 5.

Indicators of prognostic significance of the G/A rs2228480/594 polymorphism of the ESRa gene in the study groups

Group	Se	Sp	AUC	OR (95% CI)	χ2	P
Main group	0.27	0.84	0.55	0.955-3.285	3.4	0.07
Symp UF, n=53	0.28	0.84	0.56	0.856- 3.61	2.4	0.1
Asymp UF,n=49	0.26	0.84	0.54	1.017-4.234	4.1	0.04

The total prognostic significance of the G/A rs2228480/594 ESRa receptor genes for predicting the development of UF is >0.5 (AUC=0.55), which means a tendency to predict the studied receptor gene in the development of UF with a specificity of 84%.

Conclusion. Thus, the polymorphic marker rs2228480/594 of the ESRa gene in the studied women is associated with the development of UF, but clinical symptoms are not always manifested in women with abnormal homozygous genotypes. This makes it possible to assume that women with UF have a tendency to increase the frequency of carrying "mutant" alleles and genotypes for the studied genes compared with women from the population sample. The results of the study of the main characteristics of polymorphic variants of the studied genes in women with UF and a control sample showed a statistically significant increase in the risk of developing this disease with the carrier of the heterozygous unfavorable genotype G/A (OR=1.7; 95% CI 0.8402-3.632) and the mutant homozygous genotype A/A rs2228480 of the ESRa gene (OR=4.3; 95% CI 0.753-24.16) had a promoter effect in relation to UF, because the chance to identify these haplotypes is statistically significantly higher in patients of the main group, which increases the risk of developing the disease from 1.7 to 4.3 times. Population analysis of the key regulatory gene UF showed the associative roles of genetic polymorphism of the ESRa receptor gene (rs2228480/594) with the development of this pathology. But to predict the risk of UF, it is not enough to evaluate polymorphic loci in isolation, which dictates the need for further clinical studies.

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POLYMORPHISM OF THE ESTROGEN RECEPTOR GENE IN THE IN WOMEN WITH UTERINE FIBROIDS

Yuldasheva D.Y., Irnazarova D.Kh., Kayumova D.T., Irnazarov A.A., Parvizi N.I.

Uterine fibroid is one of the most common benign tumors in women in the structure of gynecological diseases. Despite its prevalence, fibroid remains a relatively understudied nosology. We examined 200 women of reproductive and premenopausal age at the Multidisciplinary Clinic of the Tashkent Medical Academy from 2018-2020. The women were divided into groups: the main group (n=102) - with symptomatic (n=53) and asymptomatic (n=49) fibroid and the control group (n=98). All women underwent general clinical and molecular genetic tests. The polymorphic marker rs2228480/594 of the ESRα gene was associated with the development of uterine fibroid in the examined women.

Key words: *uterine fibroid, polymorphism, ESRα receptor gene.*