АКУШЕРСТВО И ГИНЕКОЛОГИЯ

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CATECHOL-O-METHYLTRANSFERASE POLYMORPHISM (VAL158MET) IN WOMEN WITH UTERINE LEIOMYOMA AND ADENOMYOSIS

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The aim of this research was to study the frequency of polymorphic variants Val158Met (rs4680) of catechol-O-methyltransferase (COMT) gene in patients with uterine leiomyoma. A polymerase chain reaction was performed to figure out the frequency of polymorphic alleles of COMT gene in 54 patients with uterine leiomyoma and 103 women from the general population. It is shown that the presence of the genotype G/G of the COMT gene is associated with 2.5 times-increased risk of uterine leiomyoma (RR 2,44, Cl95: 1,168-5,103), whereas the genotype A/A is not associated with the development of leiomyoma. At the same time, a comparative analysis of the genotypes frequencies of the COMT gene polymorphism between groups with different combinations of hyperplastic processes of reproductive system (uterine leiomyoma, adenomyosis, hyperplastic processes of endometrium), showed no statistically significant differences. Refs 26. Tables 3.

Keywords: fibroids, leiomyoma, adenomyosis, hyperplastic process of endometrium, catechol-o-methyltransferase.

ВАРИАНТЫ ПОЛИМОРФИЗМА ГЕНА КАТЕХОЛ-О-МЕТИЛТРАНСФЕРАЗЫ (VAL158MET) У ЖЕНЩИН С МИОМОЙ МАТКИ И АДЕНОМИОЗОМ

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Целью работы явилось изучение частоты полиморфных вариантов гена катехол-о-метил трансферазы (COMT) Val158Met (rs4680) у больных с миомой матки. Методом полимеразной цепной реакции исследованы частоты полиморфных аллелей гена COMTу 54 больных с миомой матки и у 103 человек из популяционной выборки. Показано, что наличие генотипа G/G по гену COMT связано с повышением риска развития миомы в 2,5 раза (OR2,44, Cl95:1,168-5,103), тогда как генотип A/A не ассоциирован с развитием лейомиомы. В то же время, сравнительный анализ частоты полиморфных вариантов гена COMT у женщин с различными сочетаниями гиперпластических заболеваний органов репродуктивной системы (миома матки, аденомиоз, гиперпластические процессы эндометрия) не выявил статистически достоверных отличий. Библиогр. 26 назв. Табл. 3.

Ключевые слова: миома матки, лейомиома, аденомиоз, гиперпластические процессы эндометрия, катехол-о-метил трансфераза.

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Introduction

Diseases associated with hyperplastic processes in the female reproductive system have a multifactorial mode of inheritance. For their origin and development there must be a combined influence of genetic and environmental factors.

Genetic predisposition to the disease is mainly formed as a result of the combination of alleles of several genes that may be involved in its development and pathological manifestations [8, 9, 15, 21].

The frequency of reproductive system diseases caused by hyperplastic processes is growing steadily. According to various studies the frequency of uterine leiomyoma may reach 77% [2, 3, 14, 19].

Among gynecological patients, 50–70% are subject to surgical removal of uterine leiomyoma (20, 24). The conservative (myomectomy) surgery is not possible or appropriate in all cases, and the loss of the reproductive organ causes severe social and psychological trauma for women. The problem is compounded by the fact that the largest group among operated patients (24–26.8%) consists of women of reproductive age [2, 14].

The gene of catechol-o-methyltransferase (*COMT*) encodes a protein responsible for the metabolism of steroid hormones, and changes in the structure of the gene in their turn can cause changes in steroid metabolism, resulting in hormonal disorders. The aim of this work was to study the frequency of polymorphic variants (Val158Met) of the catechol-O-methyltransferase (*COMT*) gene in patients with uterine leiomyoma, including its combination with adenomyosis and analysis of their possible association with clinical manifestations of hyperplastic processes in the female reproductive system.

Methods

Polymerase chain reaction was performed to study the frequency of polymorphic alleles of COMT gene in 54 patients with uterine leiomyoma and 103 women from the general population. All women were divided into 2 groups:

The group of the control population included 103 women. The sample population involved women of the same race, place of birth and residence confined to a single region. The mean age in that group was 36,54 ± 3,64 years (range 22 to 50 years).

The criteria for inclusion in the control group for this study were:

1) age: older than 18 years
2) gender: female
3) race: caucasian
4) region of residence: the north-west Russia
5) voluntary participation in the study.

The main group included 50 women with uterine leiomyoma, aged from 27 years to 66 years (mean age was 40,0 ± 5,36 years). The mean duration of the disease (uterine leiomyoma) before surgery was 6,3 years (from 1 year up to 8 years). Indications for surgery were the rapid growth of uterine leiomyoma, pregnancy planning, infertility, the presence of ovarian comorbidity, chronic pelvic pain syndrome. Conservative surgery (myomectomy) was performed in 38 patients (76%). Total hysterectomy or supracervical amputation were performed in 12 women (24%).

Patient examination included a clinical-anamnestic data, ultrasound examination of the pelvic organs, histological analyses of surgical materials. The diagnosis in all cases was verified morphologically.

Age of menarche in the main group ranged from 11 to 16 years, with the mean age of 13,05 ± 0,55 years. In 47 of them menstrual period by the time of surgery was regular (from 26 to 32 days), 8 women noticed menstrual disorders: breakthrough (intermenstrual) bleeding (1 case), algomenorrhea (2); hyperpolymenorrhea (5). All women with hyperpolymenorrhea had multiple uterine leiomyoma.

Infertility was diagnosed in 19 cases: primary infertility (6), secondary infertility (13). 32 women had parturition in anamnesis (64%): pregnancy and delivery before the revelation of the disease (18), had the diagnosis before pregnancy (7), pregnancy after conservative myomectomyn (2).

Two women with a diagnosis of submucous myoma had spontaneous abortion before 12 weeks of gestation. Ectopic pregnancies occurred in 2 women with submucous-intramural localization of leiomyoma.

Multiple uterine leiomyoma was detected in 16 women (32%). The most frequent form of the disease was subserous in 72,2 ± 0,1% of cases.

The results of a histological examination of postoperative material revealed a combination of uterine leiomyoma and adenomyosis in 15 cases, a combination of uterine leiomyoma with hyperplastic process of endometrium in 8 cases.

DNA samples were prepared by standard methods from peripheral blood lymphocytes taken for Polymerase Chain Reaction analysis. The volume of the mixture for the amplification was 25 mcL, it included 15 nM of each primer, 67 mM Tris-HCl, pH 8.8, 16.6 mM ammonium sulfate, 6.7 mM MgCl2, 6,7 mM EDTA, 10 mM mercaptoethanol, 170 ug BSA, 1 0 mM each dNTP, and 1U Taq-DNA polymerase (manufactured by "Bion", Moscow).

To amplify COMT gene fragments, the following PCR conditions were used: after denaturation (94°C, 7 min.) 30 cycles of amplification were performed at: 1) 94°C, 40sec, 2) 55°C, 40sec, 3) 72°C, 1 min. (modified oligonucleotides used with the creation of a restriction site: F 5' — CGGATGGTGGATTTCGCTcG- 3'; R 5' — ACTATCACCAGGCCCCTCAG — 3').

To identify the allele of the COMT gene cleavage of obtained PCR product with the restriction enzyme BstFN1 was performed, restriction products were exposed to electrophoresis in a 7.5% polyacrylamide gel followed by staining with ethidium bromide and visualization under ultraviolet light.

Statistical analysis was performed using the computer program "GraphPadInStat, version 3.05,32." When comparing the individual frequencies of genotypes Fisher's test was used, when comparing groups — standard test χ^2. Relative Risk (RR) of the disease in a particular genotype was calculated by the standard formula RR= $a / b \times d / c$, where a and b are the number of patients with and without a mutant genotype, and c and d are the number of persons in the control group, with or without mutant genotype, respectively. RR was set with 95% confidence intervals. Confidence limits were calculated according to formulas RRmin = $RR^{(1-1,96/\sqrt{\chi 2})}$ and RRmax = $RR^{(1+1,96/\sqrt{\chi 2})}$.

Results

We studied Val158Met polymorphism of the COMT gene. Firstly, we analyzed the alleles' frequency of COMT polymorphic variants in patients with uterine leiomyoma and women from a population control sample (Table I).

Table I. **The frequency of alleles of *COMT* gene in the group of women with uterine leiomyoma and women from a population sample control**

	Womenwithleiomyoma		**Populationsample**	
Allele	**N**	**h±S_h**	**N**	**h±S_h**
A	48	44±4,8	106	51±3,5
G	60	56±4,8	100	49±3,5
	108	**100**	**206**	**100**

The frequency of allele A in the group with uterine leiomyoma was 44% (48/108), while in a population sample it was 50% (106/206), the frequency of the G allele in the group with uterine leiomyoma and in a population sample were 56% (60/108) and 49% (100/206), respectively. Comparative analysis of the *COMT* gene allele frequencies between the group of women with uterine leiomyoma and women from a population sample revealed no statistically significant difference (χ^2 = 1,394, p = 0,2).

Secondly, we analyzed the frequency of the gene COMT genotypes in patients with uterine leiomyoma and women from a population sample (Table II).

Table II. **The frequencies of genotypes of *COMT* gene in a group of women with uterine leiomyoma and women from a population sample**

	Womenwithleiomyoma		**Populationsample**		**RR (CI95%)**	***p***
Genotype	**N**	**%**	**N**	**%**		
A/A	14	26	23	22	0,82 0,382-1.766	0,69
A/G	20	37	60	58	2,37 1,205-4,669	0,01
G/G	20	37	20	20	2,44 1,168-5,103	0,02
	54	**100**	**103**	**100**		

The frequency distribution of *COMT* genotypes was significantly different between the group of women with uterine leiomyoma and control (χ^2 = 7,64 p = 0,02 df2). Genotype A/A was detected in 14 samples (26%) in the group of women with uterine leiomyoma and in 23 samples (22%) in the control group. Genotype A/G in the group of women with uterine leiomyoma was detected in 20 cases (37%) and in a population sample in 60 cases (58%). The number of patients with genotype G/G in the group of women with uterine leiomyoma was 20 (37%) and in a population sample in 20 people (20%) (Table II).

A significant increase of the frequency of the genotype G/G in the main group was revealed (p = 0,02), whereas the frequency of the genotype A/G was significantly increased

in the control group (p = 0,01). Genotype A/A was detected with equal frequency in both groups of women.

To identify characteristics of the distribution of *COMT* genotypes' frequencies in women with a combination of hyperplastic processes of the uterus further analysis was performed (Table III).

Table III. **The frequencies of genotypes of *COMT* gene in a group of women with uterine leiomyoma in combination with adenomyosis and endometrial hyperplasia**

Genotype	A/A		A/G		G/G	
	n	$h \pm S_h$(%)	n	$h \pm S_h$(%)	n	$h \pm S_h$(%)
Womenwithleiomyoma	6	26±9,1	7	30±9,6	10	43,0±10,3
Leiomyoma+ genitalendometriosis	5	33±12,1	6	40±12,6	4	27±11,5
Leiomyoma+hyperplastic processes of endometrium	1	12,5±11,7	3	37,5±17,1	4	50±17,7

As a result of analysis, we identified that the genotype G/G of *COMT* gene was detected with the same frequency in the group with isolated uterine leiomyoma, and in women with a combination of uterine leiomyoma and other uterine hyperplastic processes.

Discussion

Genetic aspects in etiopathogenesis of female reproductive hyperplastic processes are based on the changes in the expression of genes associated with the nosological form of the disease or the appearance of polymorphic gene variants (multiple mutations), which can change their functional activity.

It is known that a lot of gene products are involved in the metabolism of estrogens, including isolated genes encoding the enzymes of the first and second phases of detoxification (CYP1A1, CYP1B1, CYP3A4, *COMT*, GST) [10, 11, 19, 22].

Catechol-O-methyltransferase catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholamines, thus participating in the catabolism of catecholamines and catechol-estrogen drugs used in the treatment of hypertension, asthma and Parkinson's disease. There are two basic ways to inactivate catechol-estrogens. The first way — the conjugation with glutathione of catechol-estrogen quinones. In the regulation of this process the enzyme glutathione-S-transferase is involved, it is encoded by a superfamily of genes located on different chromosomes.

Another way to inactivate catechol-estrogens is performed by methylation with the help of the enzyme catechol-O-methyltransferase.

The gene, encoding the protein of catechol-O-methyltransferase, is mapped on the long arm of chromosome 22 in the region 22q11.21.

By methylation of 2-OH-estradiol Catechol-O-methyltransferase increases the concentration of 2-methoxyestradiol (2-MeO-E2), which in its turn has anti-proliferative, cytostatic activities, as well as reducing the possibility of damage to the DNA that is also a part of antitumor activity.

On the other hand catechol-O-methyltransferase converts 2-OH-estrogen (2OHE2) in 2-methoxyestrogen. 2 OH-estrogen in many tissues acts as an antiestrogen.

Transversion from G to A in fourth exon of *COMT* gene is converted in replacement of amino acid valine to methionine at position 158 of the protein, thus determining polymorphism of this gene. This polymorphic variant of the *COMT* gene is functionally significant. In individuals with genotype A/A enzymatic activity is reduced (13), whereas in genotype G/G catechol-O-methyltransferase will effectively convert 2-OH-estrogen to its methylated form, and thus reduces the amount of antiestrogen effects created by a high estrogen background. Thus, people with genotype A/A are characterized by low estrogen background (1). According to our data, the A /A genotype of *COMT* gene is not associated with the development of leiomyoma in the main group of women. The presence of the genotype G/G increases the risk of uterine leiomyoma by 2.5 times (RR 2,44, Cl95: 1,168-5,103). Research of the possible association of uterine leiomyoma and *COMT* gene polymorphisms were performed by German and Austrian authors (7). According to them, a polymorphic variant of Val158Met is not associated with uterine leiomyoma. While Al-Hendy A et al. (1) showed that the genotype G/G (Val\Val) is associated with the development of uterine fibroids, which is in accord with our data. One can assume that in women with genotype G/G of gene *COMT*, catechol-O-methyltransferase will effectively convert 2-OH-estrogen to its methylated form, thus reducing the amount of anti-estrogen, i.e. creating a high estrogen background. Women with this genotype, that means the high activity of catechol-O-methyltransferase have a 2.5 times greater risk of uterine fibroids.

The contradiction of our results to some of the literature allows us to conclude that there is a necessity to conduct further studies of the issue. However, it should be recognized that the final conclusion about the connection *COMT* gene polymorphism with uterine fibroids can be made only after a detailed description of the molecular mechanisms of the origin and development of uterine leiomyoma.

In the comparative analysis of the genotype frequencies of *COMT* gene Val158Met polymorphism between groups with different combinations of uterine leiomyoma with other hyperplastic processes, statistically significant differences were not found.

Thus, we can say that there is a common fragment of the pathogenesis of hyperplastic processes of the uterus associated with the metabolism of estrogen, due to *COMT* gene polymorphism.

In case of confirming these results obtained with a greater number of observations, *COMT* gene polymorphism analysis can be recommended as a prognostic test to estimate the risk of development of hyperplastic processes of myometrium, including uterine leiomyoma.

Declaration of Interеststatement

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