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Research Article

Bioinformatic Analysis of candidate genes for Endometrial Hyperplasia

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ABSTRACT.

Endometrial hyperplasia is an abnormal diffuse or local proliferation of glandular and stromal constituents of endometrium with predominant affection of glandular structures. According to literature data, frequency of this abnormality is 15-50% among all the gynecological disorders. Women with endometrial hyperplasia have an elevated risk of endometrial carcinoma. Investigation sampling comprised 1501 women: 253 patients had endometrial hyperplasia and 981 women were from the control group. In the group under study, it was performed typing seven molecular and genetic markers: *ESR1* (rs1884051), *BSX* (rs6589964), *CD40LG* (rs5930973), *FTO* (rs9939609), *PLCL1* (rs12617311), *INHBA* (rs1079866), *UGT2B4* (rs13111134). Article concerns data of the bio-informational analysis of polymorphic options of genes at patients with endometrial hyperplasia and women in control group. There were determined that combination of four genetic variants rs12617311 with rs1079866 with rs13111134 and rs9939609 (OR=0.73) is protective factor of endometrial hyperplasia, and combination genetic markers rs1884051 with rs6589964 with rs5930973 and rs9939609 (OR=2.14) is risk factor for endometrial hyperplasia in the women of Russia Central Region.

Keywords: endometrial hyperplasia, genetic polymorphism, bioinformatics.

INTRODUCTION.

Endometrial hyperplasia is a pre-cancerous, non-physiological, non-invasive proliferation of the endometrium that results in increased volume of endometrial tissue with alterations of glandular architecture (shape and size) and endometrial gland to stroma ratio of greater than 1:1 (Krivoshei I.V. et al., 2015, Chandra V. et al., 2016, Yuk J.-S. et al., 2016). Endometrial hyperplasia is defined as abnormal proliferation of the uterine endometrial glands (Ponomarenko I.V. et al., 2016a).

Endometrial hyperplasia has a significant place in the structure of gynecological morbidity in women of reproductive age and is one of the most frequent causes of hospitalization in gynecology hospital (10% to 18%) (Byun J. M. et al., 2015, Ponomarenko I.V. et al., 2016b). Women with endometrial hyperplasia have an elevated risk of endometrial carcinoma, and like endometrial carcinoma (Chen Y-L et al., 2013, Kadirogullari P. et al., 2015). Endometrial hyperplasia has been classified into 3 main types: simple hyperplasia, characterized by minimal endometrial glandular crowding and with low risk of progression to endometrial carcinoma; complex hyperplasia, characterized by greater endometrial glandular crowding and intermediate risk of progression; and atypical hyperplasia, comprised of endometrium with complex glandular crowding and/or cytologic atypia and the greatest risk of endometrial carcinoma progression (Boyraz G.et al., 2016). The most common symptom of endometrial hyperplasia is abnormal uterine bleeding including, menorrhagia, intermenstrual bleeding, postmenopausal bleeding, and irregular bleeding when on hormone replacement therapy (Orbo A. et al., 2016). Now it is known that polymorphisms of several genes are important in the formation of disposition to the development of endometrial

hyperplasia. However, the results of studies on the role of candidate genes in the formation of endometrial hyperplasia are controversial in different populations.

MATERIALS AND METHODS.

There was performed analysis of the observation data for 1501 persons: 520 patients with endometrial hyperplasia and 981 females from the reference panel. The patients and reference panels included Russian women, natives of the Central region of Russia and not having family ties among themselves. Clinical laboratory examination of the patients was performed at the gynecology department of the perinatal center of the Bishop Ioasaf Belgorod Regional Clinical Hospital. The examination of patients was carried out on the basis of the gynecology department of the perinatal centre of Belgorod region clinical hospital of Josaphat the Sanctifier. All the patients with endometrial hyperplasia and the control group samples had typing of seven molecular and genetic markers:

ESR1 c.1096+17636G>A (rs1884051), *BSX* g.122870683A>C (rs6589964), *CD40LG* c.157-277G>A (rs5930973), *FTO* c.46-23525T>A (rs9939609), *PLCL1* g.198767841G>A (rs12617311), *INHBA* g.41430495C>G (rs1079866), *UGT2B4* c.594+346C>T (rs13111134).

Venous blood samples with the volume of 8-9 ml drawn from the ulnar vein of the proband were used as a test material. Genomic DNA extraction from peripheral blood was performed by the standard method of phenol-chloroform extraction from frozen venous blood samples (Miller S. A. et al., 1988). Analysis of the examined loci was carried out by the method of polymerase chain reaction of DNA synthesis with use of oligonucleotide primers and probes. Estimation of role of the studied genetic variants combinations in contraction of endometrial hyperplasia is performed using the software APSampler using Markov chains Monte Carlo technique and Bayesian distribution-free statistics. In order to minimize type I errors, Bonferroni correction (pcor) and permutation test (pperm) were used for multiple comparisons (Favorov, A. V. et al., 2005).

RESULTS AND DISCUSSION.

After examination of 520 women with endometrial hyperplasia and 981 women from the control group, it was determined, that the control group is completely commeasurable with sampling of cases with genital endometriosis by gender, age, nationality and place of birth, and by height and weight ($p>0.05$). Main characteristics of the studied groups are given in the Table 1.

Table 1: Characteristics of the subjects from the case and control groups.

Characteristics	Cases	Controls
Total	520	981
Age, yrs	41.78 ±10.04	40.73 ±8.60
Weight, kg	61.5±2.9	64.3±3.5
Height, cm	163.7±3.1	167.4±3.8

Examination of alleles concentration of genes polymorphic markers under study showed that for all the examined locuses in the group of patients with genital endometriosis and in population sampling, empiric genotype distribution corresponded to the expected one at Hardy-Weinberg equilibrium ($p>0.05$) (Table 2).

Polymorphism	Studied groups	Minor allele	MAF (%)	HWE	
				χ^2	p
ESR1 c.1096+17636G>A (rs1884051)	Case	*G*	0.39	3,25	>0.05
ESR1 c.1096+17636G>A (rs1884051)	Control	*G*	0.35	2,96	>0.05
BSX g.122870683A>C(rs6589964)	Case	*A*	52.23	2.42	>0.05
BSX g.122870683A>C(rs6589964)	Control	*A*	49.25	2.49	>0.05
CD40LG c.157-277G>A (rs5930973)	Case	*A*	0.19	0.89	>0.05
CD40LG c.157-277G>A (rs5930973)	Control	*A*	0.15	0.97	>0.05
FTO c.46-23525T>A (rs9939609)	Case	*A*	0.49	0.40	>0.05
FTO c.46-23525T>A (rs9939609)	Control	*A*	0.46	0.32	>0.05
PLCL1 g.198767841G>A (rs12617311)	Case	*A*	0.39	0.35	>0.05
PLCL1 g.198767841G>A (rs12617311)	Control	*A*	0.35	0.43	>0.05
INHBA g.41430495C>G (rs1079866)	Case	*C*	0.21	1,12	>0.05
INHBA g.41430495C>G (rs1079866)	Control	*C*	0.19	1,25	>0.05
UGT2B4 c.594+346 G>A (rs13111134)	Case	*A*	0.31	1,67	>0.05
UGT2B4 c.594+346 G>A (rs13111134)	Control	*A*	0.27	1,59	>0.05

Table 2: Summary information about the studied polymorphisms.

Notes: MAF, minor allele frequency; Hardy – Weinberg equilibrium. P values were calculated using the $\chi 2$ test.

While using bio-informational approaches, is was determined that combination of four genetic variants G *ESR1* (rs1884051) with C *BSX* (rs6589964) with A *CD40LG* (rs5930973) and A *FTO* (rs9939609) in the group of cases with endometrial hyperplasia (5.03%) is much more often (2.2 times more) than in the control group (2.29%, pbonf=0.04, pperm=0.008). These data testify about a great contribution of combination of polymorphicc genes variants rs1884051 and rs6589964 and rs5930973 and rs9939609 to endometrial hyperplasia (OR=2.14, CI 1.19-3.86).

The estrogen receptor 1 (*ESR1*) gene encodes an estrogen receptor, a ligand-activated transcription factor composed of several domains important for hormone binding, DNA binding, and activation of transcription. The protein localizes to the nucleus where it may form a homodimer or a heterodimer with estrogen receptor 2 (Hewitt S. C. et al., 2016). Estrogen and its receptors are essential for sexual development and reproductive function, but also play a role in other tissues such as bone. Estrogen receptors are also involved in pathological processes including breast cancer, endometrial cancer, and osteoporosis (Jeselsohn R. et al., 2015, Okur H. S. et al., 2016). Alternative promoter usage and alternative splicing result in dozens of transcript variants, but the full-length nature of many of these variants has not been determined (Hewitt S. C. et al., 2016).

DNA binding protein *BSX* that function as transcriptional activator. Is essentiel for normal postnatal growth and nursing. Is an essential factor for neuronal neuropeptide Y and agoutirelated peptide function and locomotory behavior in the control of energy balance (Lee B. et al., 2013).

The Fat mass and obesity associated (*FTO*) gene is a nuclear protein of the AlkB elated non-haem iron and 2-oxoglutarate-dependent oxygenase superfamily but the exact physiological function of this gene is not known (Qi Q. et al., 2014). Other non-heme iron enzymes function to reverse alkylated DNA and RNA damage by oxidative demethylation. Studies in mice and humans indicate a role in nervous and cardiovascular systems and a strong association with body mass index, obesity risk, and type 2 diabetes (Harbron J. et al., 2014, Loos R. J.F. et al., 2014,).

It has been discovered that combination of genetic variants G rs12617311 (*PLCL1*) with G rs1079866 (*INHBA*) with A rs13111134 (*UGT2B4*) and A rs9939609 *FTO* occur in 20.70% of sick women, respectively, which is 1.27 times lower than that occur in control group (26.31%, pbonf=0.04, pperm=0.008). When there are these combination of polymorphic markers, pathology risk of endometrial hyperplasia is significantly lower (OR=0.73, CI 0.56-0.94).

Phospholipase C-like 1 **(***PLCL1***)** is a protein-coding gene involved in an inositol phospholipid-based intracellular signaling cascade. Shows no PLC activity to phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol. Component in the phospho-dependent endocytosis process of GABA A receptor (By similarity). Regulates the turnover of receptors and thus contributes to the maintenance of GABA-mediated synaptic inhibition (Liu Y.-Z. et al., 2008).

The inhibin beta A (*INHBA*) subunit joins the alpha subunit to form a pituitary FSH secretion inhibitor. Inhibin has been shown to regulate gonadal stromal cell proliferation negatively and to have tumor-suppressor activity. In addition, serum levels of inhibin have been shown to reflect the size of granulosa-cell tumors and can therefore be used as a marker for primary as well as recurrent disease. Because expression in gonadal and various extragonadal tissues may vary severalfold in a tissue-specific fashion, it is proposed that inhibin may be both a growth/differentiation factor and a hormone. Furthermore, the beta A subunit forms a homodimer, activin A, and also joins with a beta B subunit to form a heterodimer, activin AB, both of which stimulate FSH secretion (Tournier I. et al., 2014).

Glycosyltransferase 2 family, polypeptide B4 (*UGT2B4*) is of major importance in the conjugation and subsequent elimination of potentially toxic xenobiotics and endogenous compounds. This isozyme is active on polyhydroxylated estrogens (such as estriol, 4-hydroxyestrone and 2-hydroxyestriol) and xenobiotics (such as 4-methylumbelliferone, 1-naphthol, 4-nitrophenol, 2-aminophenol, 4-

hydroxybiphenyl and menthol) (Sun C. et al., 2011).

CONCLUSION.

Therefore the results of work allow making a conclusion that combination of four genetic variants rs12617311 with rs1079866 with rs13111134 and rs9939609 (OR=0.73) is protective factor of endometrial hyperplasia, and combination genetic markers rs1884051 *with* rs6589964 with rs5930973 and rs9939609 (OR=2.14) is risk factor for endometrial hyperplasia in the women of Russia Central Region.

Summary. Genetic polymorphisms rs12617311, rs1079866, rs13111134, rs9939609, rs1884051, rs6589964 and rs5930973 are associated with the development of endometrial hyperplasia.

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