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Intergenic Interactions and Hyperplastic Diseases of Endo- and Myometrium

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Abstract
This paper presents the results of bioinformatic analysis of three polymorphic loci in 170 women who suffer from a combination of uterine fibroids with endometrium hyperplastic processes and in 981 women of the control group without proliferative diseases of pelvic organs. It was found that the combinations of molecular genetic markers C rs673220, A rs4986938 и G rs887912 (OR=1.70) are risk factors for the development of a combination of endometrial hyperplasia and uterine myoma among women of the Central region of Russia.

Keywords: Hyperplastic Processes of the Endometrium, Uterine Myoma, Combined Pathology, Genetic Polymorphism.

Introduction

Among the gynecological incidence, the hyperplastic processes of the endometrium occupy a leading place and amount to 10-50% [Boyraz G. et al., 2016]. Endometrial hyperplasia is a pathological process affecting the epithelial and stromal components of the endometrium and is manifested by an increase in the total number of glands [Clement N.S. et al., 2016]. Atypical endometrial hyperplasia is a precancerous condition, which can cause the development of endometrial cancer in 15-45% of cases [Kadirogullari P. et al., 2015]. The risk of development of endometrial cancer in women increases significantly with age and ranges from 5 to 10% [Kim M.-J. et al., 2016].

Uterine myoma is a benign monoclonal tumor composed of the smooth muscle cells of the myometrium. The prevalence of uterine fibroids ranges from 5% to 65%, depending on age, ethnicity, geographic region and diagnostic method [Gurusamy K.S. et al., 2016]. According to the literature, uterine fibroids are most often observed in women of reproductive age (about 40%) [Khan A.T. et al., 2014] and reaches a peak at 50 years [Zimmermann A. et al., 2012]. According to various data, surgical interventions are performed in 25-50% of women diagnosed with uterine myoma [Gurusamy K.S. et al., 2016].

According to literature data, the combination of endometrial hyperplasia with uterine myoma occurs in 30-35% of patients, due to the common etiology, risk factors and key pathogenetic links [Tan N. et al., 2014]. The well-known risk factors for the development of proliferative diseases of the uterus include age, early menarche, late menopause, absence of pregnancy in history, obesity, diabetes, family history, lifestyle, etc. [Segars J.H. et al., 2014; Chandra V. et al., 2016]. The leading premium in the etiopathogenesis of hyperplastic processes of the endometrium and uterine myoma is set on excessive estrogen stimulation, hormone-independent proliferation, inflammation, reduced apoptosis, pathological neoangiogenesis, and cytogenetic disorders [Segars J.H. et al., 2014; Chandra V. et al., 2016].

The combined development of these diseases is of great clinical importance, since it raises significant difficulties in diagnosing and choosing the most effective method of treatment. In addition, it leads to a decrease in the quality of life of a woman.

Now it is known that polymorphisms of several genes are important in the formation of disposition to the development of hyperplastic diseases of uterus [Edwards T.L. et al., 2013; Krivoshei I.V. et al., 2015; Orbo A. et al., 2016; Pachomov S.P. et al., 2016; Ponomarenko, I.V. et al., 2016]. However, the results of studies on the role of candidate genes in the formation of hyperplastic processes of the endometrium and uterine myoma are controversial in different populations.

Objective of the research was a bioinformatic study of the involvement of three genetic polymorphisms (rs887912, rs6732220, rs4986938) in the development of a combination of endometrium hyperplastic processes with uterine myoma in women of the Central region of Russia.

Materials and Methods

In this study, patients with a combination of uterine myoma and endometrial hyperplasia (n=170) were selected from a group of 947 patients with various uterine hyperplastic processes. The sample was made on the basis of the gynecological department of the perinatal center of St. Joasaph Belgorod Regional

Clinical Hospital. Patients with a combination of uterine myoma and endometrial hyperplasia underwent a clinical, clinical and laboratory examination, ultrasound examination of pelvic organs, hysteroscopy followed by targeted diagnostic scraping of the uterine cavity and histological examination of scrapings. All patients signed an informed consent for inclusion in the study and use of the data obtained. The control group involved 981 women without proliferative diseases of the pelvic organs.

All patients with endometrial hyperplasia and uterine myoma were performed typing of three molecular-genetic markers rs887912 *FLJ30838*, rs6732220 *FSHR*, rs4986938 *ESR2*. The choice of these polymorphic markers for the study is due to their significant regulatory and expression potential (HaploReg (v.4.1.) (http://compbio.mit.edu/HaploReg)) and the important ethiopathogenetic significance of these genes for hyperplastic uterine disease [Commandeur A.E. et al., 2015].

As the material for the study we used 8-9 ml of venous blood taken from the cubital vein of a proband. A genomic DNA was isolated from peripheral blood by the method of phenol-chloroform extraction (Mathew C.G., 1985). Analysis of the investigated loci was carried out by the method of polymerase chain reaction of DNA synthesis with the use of oligonucleotide primers and probes.

Statistical processing of data was carried out using STATISTICA for Windows 6.0 and Microsoft Excel 2007 software packages. To analyze the compliance of the observed distribution of genotypes with the expected one, based on Hardy-Weinberg equilibrium, we used χ^2 test.

A bioinformatic study was performed by APSampler [http://sources.redhat.com/cygwin/], operating Markov chain Monte Carlo, and Bayesian nonparametric statistics [Favorov A., 2011].

Results and Discussion

We examined 170 female patients with the combination of uterine myoma and endometrial hyperplasia, and 981 women of the control group. The main characteristics of the study and control groups are shown in Table 1. The control group is fully comparable to the sample of patients with the combination of uterine myoma and endometrial hyperplasia by age, ethnicity, and place of birth.

Table 1 Characteristics of the Subjects from the Case and Control Groups.

Characteristics	Cases	Controls
Total	170	981
Age, yrs	45.06±8.23	39.94±9.31
Height, cm	1.66±0.07	1.65±0.06
Weight, kg	77.09±14.50	69.69±12.49
IMB	28.10±5.80	25.69±4.24

The analysis of the distribution of studied polymorphic markers of candidate genes showed that for all the studied loci in the group of patients with the combination of uterine myoma and endometrium hyperplastic processes, as well as in the control sample, the empirical distribution of genotypes corresponds to the theoretically expected one at Hardy-Weinberg equilibrium ($p>0.05$).

As a result of the bioinformatic analysis, significant differences in the concentrations of C alleles rs673220 with A rs4986938 with G rs887912 were found among the patients with a combination of endometrial hyperplasia and uterine myoma (62.87%) and the control group (49.84%). This combination of allelic variants is a risk factor for the development of endometrial hyperplasia and uterine myoma ($p=0.001$, $p_{perm} = 0.011$, OR=1.70, 95% CI 1.21-2.39).

Using HaploReg (v.4.1.) online service (http://compbio.mit.edu/HaploReg), the regulatory potential and the influence on the expression of genes of significant polymorphisms associated with the combination of endometrial hyperplasia and uterine myoma were studied. The genetic polymorphism rs6732220, localized in the intron portion of the *FHSR* gene, affects the transcription activity of this gene in the testes (p=2.81E-08). It participates in the development of menarche age (p=0.0018) [Lappalainen T. et al., 2013].

The genetic polymorphism rs887912, located 12 kb from the 3' end of the *FLJ30838* gene has a high regulatory and expressive potential and marks histone proteins within the enhancers. It should be noted that G allele (as part of the three combinations increases the risk of hyperplastic processes of the endothelium and uterine myoma) increases the affinity of the DNA motif - Hoxa5_1 (PWM = -0.6), while its reference A allele is associated with increased affinity of the Znf143_known1 motif (PWM=1.0). This genetic polymorphism is associated with a body mass index (p=2E-22) [Speliotes E.K. et al., 2010] and anthropometric characteristics at pubertal age (p=1E-10) [Berndt S.I. et al., 2013] and the age of menarche [Fernandez-Rhodez L. et al., 2013].

The polymorphic locus rs4986938, located in 3' of the end of the *ESR2* gene, marks histone proteins in the region of promoters in the cerebral cortex and blood cells. It affects the expression level of the *ESR2* gene in the left ventricle of the heart (p=9.99E-07), skin cells (p=3.86E-08) [Ardlie K.G. et al., 2015] and lymphoblast cells (p=3.65E-10) [Lappalainen T. et al., 2013]. It should be noted that this gene is most actively expressed in testes (RPKM: 1.79±0.27), adrenal glands (RPKM: 1.09±0.28), ovaries (RPKM: 0.60±0.09), lymph nodes (RPKM: 0.42±0.21), adipose

tissue (RPKM: 0.48±0.04). This genetic polymorphism is part of five DNA motifs. At the same time, the "risky" A allele increases the affinity of the regulatory motif CTCF_known1 (PWM=0.6), Pax-6_1 (PWM=11.1), RAR (PWM=0.1).

Conclusion

Thus, the results of the study allow us to conclude that the combinations of molecular genetic markers C rs673220, A rs4986938 и G rs887912 (OR=1.70) are risk factors for the development of a combination of endometrial hyperplasia and uterine myoma among women of the Central region of Russia.

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