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## MOLECULAR-GENETIC FACTORS OF GENITAL ENDOMETRIOSIS

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### Abstract

Article concerns data of the comparative analysis of polymorphic options of genes at patients with genital endometriosis and women in control group. There were determined CT genotype of rs4374421 LHCGR locus (OR=0.76) is protective factor of genital endometriosis, and genetic markers TT rs7753051 IGF2R and AA rs6589964 BSX (OR=1.32 and OR=1.38, respectively) are risk factors for genital endometriosis in the women of Russia Central Region.

**Keywords:** Genital endometriosis, Polymorphism, Genetic variations.

### Introduction

Genital endometriosis is a common, chronic, inflammatory and estrogen-dependent gynecological disease. It affects 10 to 15% of women in their reproductive years and is characterized by the implantation of endometrial (womb lining) tissue outside of the uterus (ectopic endometrium) (Bruner-Tran et al., 2013; Churnosov et al., 2015). Common locations of ectopic endometrial implantation are the pelvic peritoneum, ovaries, bowel, bladder, and less frequently, the pleural cavity, liver and kidneys. Genital endometriosis is diagnosed in 30 to 40% of women with infertility and pelvic pain (Rahmioglu et al., 2015). Women frequently experience symptoms of dysmenorrhea (painful menstruation), dyspareunia (pain during sexual intercourse), dysuria (pain during urination) and dyschezia (difficulty with defecating).

Genital endometriosis is not uncommon among adolescents. Approximately half of women under 20 years of age who have chronic pelvic pain or dyspareunia have the disease (Brosens et al., 2011). About 5% of endometriosis cases are seen in postmenopausal women, and exogenous estrogen replacement therapy is suggested to play a role.

Genital endometriosis develops as a consequence of a combination of genetic predisposition and environmental factors (Nyholt et al., 2014). In this connection a considerable number of works are dedicated to study of genetic

principles of endometriosis development (Dun et al., 2010; Trabert et al., 2011; Guo et al., 2014; Krivoshei et al., 2015). Nevertheless it is worth mentioning that the results obtained by different groups of investigators are contradictory and do not give an unambiguous answer to the question about the role of genetic factors in pathogenesis and clinical features of genital endometriosis.

## **Materials and Methods**

There was performed analysis of the observation data for 1398 persons: 403 patients with genital endometriosis and 995 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 patients with genital endometriosis were subject to pelvic organs ultrasonography, hysteroscopy with the subsequent directional biopsy of the lining of the uterus and histologic examination of the scrape, the procedures were performed by means of the common and laboratory methods of examination.

All the patients with genital endometriosis and the control group samples had typing of five molecular and genetic markers: LHCGR c.3441+36573C>T (rs4374421), BSX g.122870683A>C (rs6589964), IGF2R c.\*1941T>C (rs7753051), ESR1 c.1096+13570G>A (rs3020394), GC c.59-1155G>A (rs222020).

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 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.

Associations of alleles and genotypes of the studied DNA-markers with development of genital endometriosis were assessed by means of analysis of 2x2 contingency tables which included calculation of test with Yates' correction for continuity and odds ratio (OR) with 95% confidence interval.

## **Results and discussion**

After examination of 403 women with genital endometriosis and 995 women from the control group, it was determined, that the control group is completely commensurable 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	403	995
Age, yrs	36.05±8.06	38.2±10.1
Weight, kg	58.8±1.8	60.4±2.4
Height, cm	162.8±4.1	168.5±3.7

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).

**Table 2: Summary information about the studied polymorphisms.**

Polymorphism	Studied groups	Minor allele	MAF (%)	HWE	
				$\chi^2$	p
LHCGR c.3441+36573C>T (rs4374421)	Case	C	30.64	5.43	>0.05
LHCGR c.3441+36573C>T (rs4374421)	Control	C	31.26	0.90	>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
IGF2R c.*1941T>C (rs7753051)	Case	C	27.79	0.92	>0.05
IGF2R c.*1941T>C (rs7753051)	Control	C	31.16	3.79	>0.05
ESR1 c.1096+13570G>A (rs3020394)	Case	A	70.84	0.81	>0.05
ESR1 c.1096+13570G>A (rs3020394)	Control	A	70.35	2.57	>0.05
GC c.59-1155G>A (rs222020)	Case	C	13.24	0.10	>0.05
GC c.59-1155G>A (rs222020)	Control	C	11.43	0.38	>0.05

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

It was ascertained that the patients with genital endometriosis demonstrated high frequency of genotype TT rs7753051 IGF2R (53.10%) as compared to the reference group (46.06%,  $\chi^2=5.40$ ,  $p=0.02$ , OR=1.32, 95% CI 1.04 – 1.68).

Insulin-like growth factor 2 receptor (IGF2R) encodes a receptor for both insulin-like growth factor 2 and mannose 6-phosphate, although the binding sites for either are located on different segments of the receptor. This receptor functions in the intracellular trafficking of lysosomal enzymes, the activation of transforming growth factor beta, and the degradation of insulin-like growth factor 2.

While the 173 related mouse gene shows exclusive expression from the maternal allele, imprinting of the human gene appears to be polymorphic, with only a minority of individuals showing expression from the maternal allele (Pyun et al., 2014).

Similar results were obtained in respect to distribution of genotypes of rs6589964 BSX locus: higher frequency of AA genotype was detected (1.27 times) in the group of patients with genital endometriosis (29.21%), comparing to control group (22.96%,  $\chi^2=5.55$ ,  $p=0.02$ , OR=1.38, 95%CI 1.05-1.81).

DNA binding protein BSX that function as transcriptional activator. Is essential 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 (Elks et al., 2010).

Significant differences in genotypes concentrations between patients with genital endometriosis and control patients were observed per rs4374421 LHCGR as well: the frequency of CT genotype equaled 44.29% among the control group, which was higher than that in the patients with genital endometriosis (37.80%,  $\chi^2=4.36$ ,  $p=0.04$ , OR=0.76, 95%CI 0.59-0.98).

Luteinizing hormone/choriogonadotropin receptor (LHCGR) encodes the receptor for both luteinizing hormone and choriogonadotropin. This receptor belongs to the G-protein coupled receptor 1 family, and its activity is mediated by G proteins, which activate adenylate cyclase.

Mutations in this gene result in disorders of male secondary sexual character development, including familial male precocious puberty, also known as testotoxicosis, hypogonadotropic hypogonadism, Leydig cell adenoma with precocious puberty, and male pseudohermaphroditism with Leydig cell hypoplasia (Perry et al., 2014).

No significant differences between the groups were found regarding to allele and genotype frequencies of the of the rs3020394 ESR1 and rs222020 GC genes.

## Conclusion

Therefore the results of work allow making a conclusion that CT genotype of rs4374421 LHCGR locus (OR=0.76) is protective factor of genital endometriosis, and genetic markers TT rs7753051 IGF2R and AA rs6589964 BSX (OR=1.32 and OR=1.38, respectively) are risk factors for genital endometriosis in the women of Russia Central Region.

## References

1. Brosens, I. and G. Benagiano, 2011. Endometriosis, a modern syndrome. *Indian J Med Res*, 133(6): 581–593.
2. Elks, C. E., J. R. Perry, P. Sulem, and D. I. Chasman, 2010. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. *Nat Genet.*, 42(12): 1077–1085.
3. Dun, E.C, R.N. Taylor, and F. Wieser, 2010. *Genome Med*, 2(10):75.
4. Bruner-Tran, K. L., J. L. Herington, A. J. Duleba, H. S. Taylor, and K. G. Osteen, 2013. Medical Management of Endometriosis: Emerging Evidence Linking Inflammation to Disease Pathophysiology. *Minerva Ginecol.*, 65(2): 199–213.
5. Churnosov, M. I., O. B. Altuchova, N. A. Demakova, V. I. Evdokimov, I. N. Sorokina, 2014 a. Analysis of Involvement of Cytokine Genetic Polymorphisms in Development of Genital Endometriosis. *Research Journal of Pharmaceutical, Biological and Chemical*, №5(5): 1027-1031.
6. Guo, R, N. Zheng, S. Ding, Y. Zheng, and L. Feng, 2014. Associations between estrogen receptor-beta polymorphisms and endometriosis risk: a meta-analysis. *Diagnostic Pathology*, 9: 184.
7. Krivoshei, I. V., O. B. Altuchova, A. V. Polonikov, and M. I. Churnosov, 2015. Bioinformatic Analysis of the Liability to the Hyperplastic Processes of the Uterus. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 6(5): 1563-1566.
8. Miller, S. A., D. D. Dykes, H. F. Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic acids research*, 16(3): 1215.
9. Nyholt, D. R., A. P. Morris, S. A. Missmer, G. W. Montgomery, and K. T. Zondervan, 2014. Genetic variants underlying risk of endometriosis: insights from meta-analysis of eight genome-wide association and replication datasets. *Hum Reprod Update*, 20(5): 702–716.
10. Perry, J.R., F. Day, C.E. Elks, P. Sulem, D.J. Thompson, T. Ferreira, 2014. Parent-oforigin-specific allelic associations among 106 genomic loci for age at menarche. *Nature*, 514:92-7.

11. Pyun, J.A., S. Kim, N.H. Cho, I. Koh, J.Y. Lee, C. Shin, 2014. Genome-wide association studies and epistasis analyses of candidate genes related to age at menarche and age at natural menopause in a Korean population. *Menopause*, 21:522-9.
12. Rahmioglu, N. , S. Macgregor, A. W. Drong, Å. K. Hedman, H. R. Harris, J. C. Randall, I. Prokopenko, 2015. Genome-wide enrichment analysis between endometriosis and obesity-related traits reveals novel susceptibility loci. *Hum Mol Genet.*, 24(4): 1185–1199.
13. Trabert, B., S. M. Schwartz, U. Peters, A. J. De Roos, C. Chen, D. Scholes, and V. L. Holt, 2011. Genetic variation in the sex hormone metabolic pathway and endometriosis risk: an evaluation of candidate genes. *Fertil Steril.*, 96(6): 1401–1406.

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