THE ROLE OF GENE-GENE INTERACTIONS IN THE FORMATION OF THE GENITAL ENDOMETRIOSIS

Irina V. Ponomarenko, Vladimir F. Kulikovskiy, Evgeniy N. Krikun, Alexander A. Dolzhikov, Olga Yu. Bushueva and Alexey V. Polonikov

1Belgorod State University, 308015, Belgorod, Pobeda Street, 85, Russia
2Kursk State Medical University, 305041, ul. Karla Marksa, 3, Kursk, Kursk Oblast, Russia

Abstract:
The article highlights the bioinformatics analysis data of seven polymorphous loci among 395 patients with genital endometriosis and 981 women from the control group. It was found that the increased risk of genital endometriosis in women of Russia Central region is connected with the combination of alleles C rs2252673 with T rs12444979 with G rs6732220 with G rs7538038 (OR=4.81), and the protective effect have the combination of the following molecular genetic markers: A rs2013573 with T rs2288696 with A rs10980926 (OR = 0.41).

Keywords: genital endometriosis, polymorphism, genetic variations, bioinformatics.

Corresponding author:
Irina V. Ponomarenko,
Belgorod State University,
308015, Belgorod, Pobeda Street,
85, Russia.
e-mail: Ponomarenko_i@bsu.edu.ru

Please cite this article in press as Irina V. Ponomarenko et al., The Role of Gene-Gene Interactions in the Formation of the Genital Endometriosis, Indo Am. J. P. Sci, 2017; 4(10).
INTRODUCTION:
Genital endometriosis is an estrogen-dependent inflammatory disease that is characterized histologically by the presence of endometrial glands and stroma outside the uterine cavity [1]. Genital endometriosis is found predominantly in women of childbearing age. Approximately 10-15% of women of reproductive age have been reported to suffer from this condition [2]. The mean age at diagnosis is 25–29 years [3]. Women with genital endometriosis frequently report pain symptoms, including chronic pelvic pain, painful menstrual periods, and pain with intercourse.
Risk factors of genital endometriosis include age, increased exposure to menstruation (shorter cycle length, longer duration of flow and nulliparity) and other factors related to estrogen levels, including decreased body mass index and smoking history [4]. Endometrial lesions are primarily located on the pelvic peritoneum and ovaries but can also be found in the pericardium, pleura, lung parenchyma, and even the brain. Implants can result in substantial morbidity, including pelvic adhesions, pain, fatigue, bowel disorders, and infertility requiring extensive and sometimes ineffective medical and surgical treatments [5,6]. It affects deeply and negatively woman’s quality of life, contributing not only to suffering but also to marital and family problems, to problems related to the achievements of work tasks, and overall to disability in woman’s role in modern society. The causes of the condition are largely unknown, but are likely to be complex, involving multiple environmental and genetic factors.

MATERIALS AND METHODS:
There was performed analysis of the observation data for 1376 persons: 395 patients with genital endometriosis 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 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 seven molecular and genetic markers: INSR c.2267+90G>C (rs2252673), GPRC5B g.19933600C>T (rs12444979), FSHR c.375-5096C>G (rs6732220), KISS1 c.103+8766A>G (rs7538038), UGT2B4 c.1002+679G>A (rs2013573), FGFR1 c.359-272C>T (rs2288696), ZNF483 c.501+391A>G (rs10980926).
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 [7].

RESULTS AND DISCUSSION:
After examination of 395 women with genital endometriosis and 981 women from the control group, it was determined, that the control group is completely comparable 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.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>395</td>
<td>981</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>34.75±6.1</td>
<td>36.8±9.1</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>59.4±1.6</td>
<td>61.8±3.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>163.6±3.5</td>
<td>167.3±2.3</td>
</tr>
</tbody>
</table>
Table 2: Summary information about the studied polymorphisms.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Studied groups</th>
<th>Minor allele</th>
<th>MAF (%)</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INSR c.2267+90G&gt;C</strong> (rs2252673)</td>
<td>Case</td>
<td>C</td>
<td>0.39</td>
<td>3.25 &gt;0.05</td>
</tr>
<tr>
<td><strong>INSR c.2267+90G&gt;C</strong> (rs2252673)</td>
<td>Control</td>
<td>C</td>
<td>0.35</td>
<td>2.96 &gt;0.05</td>
</tr>
<tr>
<td><strong>GPRC5B g.19933600C&gt;T</strong> (rs12444979)</td>
<td>Case</td>
<td>T</td>
<td>52.23</td>
<td>2.42 &gt;0.05</td>
</tr>
<tr>
<td><strong>GPRC5B g.19933600C&gt;T</strong> (rs12444979)</td>
<td>Control</td>
<td>T</td>
<td>49.25</td>
<td>2.49 &gt;0.05</td>
</tr>
<tr>
<td><strong>FSHR c.375-5096C&gt;G</strong> (rs6732220)</td>
<td>Case</td>
<td>G</td>
<td>0.19</td>
<td>0.89 &gt;0.05</td>
</tr>
<tr>
<td><strong>FSHR c.375-5096C&gt;G</strong> (rs6732220)</td>
<td>Control</td>
<td>G</td>
<td>0.15</td>
<td>0.97 &gt;0.05</td>
</tr>
<tr>
<td><strong>KISS1 c.103+876A&gt;G</strong> (rs7538038)</td>
<td>Case</td>
<td>G</td>
<td>0.49</td>
<td>0.40 &gt;0.05</td>
</tr>
<tr>
<td><strong>KISS1 c.103+876A&gt;G</strong> (rs7538038)</td>
<td>Control</td>
<td>G</td>
<td>0.46</td>
<td>0.32 &gt;0.05</td>
</tr>
<tr>
<td><strong>UGT2B4 c.1002+679G&gt;A</strong> (rs2013573)</td>
<td>Case</td>
<td>A</td>
<td>0.39</td>
<td>0.35 &gt;0.05</td>
</tr>
<tr>
<td><strong>UGT2B4 c.1002+679G&gt;A</strong> (rs2013573)</td>
<td>Control</td>
<td>A</td>
<td>0.35</td>
<td>0.43 &gt;0.05</td>
</tr>
<tr>
<td><strong>FGFR1 c.359-272C&gt;T</strong> (rs2288696)</td>
<td>Case</td>
<td>T</td>
<td>0.21</td>
<td>1.12 &gt;0.05</td>
</tr>
<tr>
<td><strong>FGFR1 c.359-272C&gt;T</strong> (rs2288696)</td>
<td>Control</td>
<td>T</td>
<td>0.19</td>
<td>1.25 &gt;0.05</td>
</tr>
<tr>
<td><strong>ZNF483 c.501+391A&gt;G</strong> (rs10980926)</td>
<td>Case</td>
<td>G</td>
<td>0.31</td>
<td>1.67 &gt;0.05</td>
</tr>
<tr>
<td><strong>ZNF483 c.501+391A&gt;G</strong> (rs10980926)</td>
<td>Control</td>
<td>G</td>
<td>0.27</td>
<td>1.59 &gt;0.05</td>
</tr>
</tbody>
</table>

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

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).

As a result of bioinformatics analysis it was found, that combination of four genetic variants C **INSR** (rs2252673) with T **GPRC5B** (rs12444979) with G **FSHR** (rs6732220) and G **KISS1** (rs7538038) in the group of cases with genital endometriosis (5.21%) is much more often (4.6 times more) than in the control group (1.13%, p=0.000023). These data testify about a great contribution of combination of polymorphic genes variants rs2252673 with rs12444979 with rs6732220 and rs7538038 to genital endometriosis (OR=4.81, CI 2.28-10.13).

Insulin receptor (**INSR**) encodes a member of the receptor tyrosine kinase family of proteins. The encoded preproprotein is proteolytically processed to generate alpha and beta subunits that form a heterotetrameric receptor. Binding of insulin or other ligands to this receptor activates the insulin signaling pathway, which regulates glucose uptake and release, as well as the synthesis and storage of carbohydrates, lipids and protein. Mutations in this gene underlie the inherited severe insulin resistance syndromes including type A insulin resistance syndrome, Donohue syndrome and Rabson-Mendenhall syndrome. Alternative splicing results in multiple transcript variants [8].

G protein-coupled receptor, class C, group 5, member B (**GPRC5B**) the protein encoded by this gene is a member of the type 3 G protein-coupled receptor family. Members of this superfamily are characterized by a signature 7-transmembrane domain motif. The specific function of this protein is unknown; however, this protein may mediate the cellular effects of retinoic acid on the G protein signal transduction cascade [9].

Follicle stimulating hormone receptor (**FSHR**) encoded by this gene belongs to family I of G-
protein coupled receptors. It is the receptor for follicle stimulating hormone and functions in gonad development. Mutations in this gene cause ovarian dysgenesis type 1, and also ovarian hyperstimulation syndrome. Alternative splicing results in multiple transcript variants [10].

The metastasis-suppressor gene (KISS1) is a metastasis suppressor gene that suppresses metastases of melanomas and breast carcinomas without affecting tumor igenicity. The encoded protein may inhibit chemotaxis and invasion and thereby attenuate metastasis in malignant melanomas. Studies suggest a putative role in the regulation of events downstream of cell-matrix adhesion, perhaps involving cytoskeletal reorganization. A protein product of this gene, kisspeptin, stimulates gonadotropin-releasing hormone (GnRH)-induced gonadotropin secretion and regulates the pubertal activation of GnRH neurons. A polymorphism in the terminal exon of this mRNA results in two protein isoforms. An adenosine present at the polymorphic site represents the third position in a stop codon. When the adenosine is absent, a downstream stop codon is utilized and the encoded protein extends for an additional seven amino acid residues [11].

It has been discovered that combination of genetic variants A UGT2B4 (rs2013573) with T FGFR1 (rs2288696) and A ZNF483 (rs10980926) occur in 3.63% of sick women, respectively, which is 2.32 times lower than that occur in control group (8.41%, p=0.0008). When there are these combination of polymorphic markers, pathology risk of genital endometriosis is significantly lower (OR=0.41, CI 0.23-0.73).

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-hydroxyestradiol) and xenobiotics (such as 4-methylumbelliferone, 1-naphthol, 4-nitrophenol, 2-amino phenol, 4-hydroxybiphenyl and menthol) [11].

Zinc finger protein 483 (ZNF483) may be involved in transcriptional regulation [12].

Fibroblast growth factor receptor 1 (FGFR1) encoded by this 174 gene is a member of the FGFR family, where amino acid sequence is highly conserved between members and throughout evolution. FGFR family members differ from one another in their ligand affinities and tissue distribution. A full-length representative protein consists of an extracellular region, composed of three immunoglobulin-like domains, a single hydrophobic membrane-spanning segment and a cytoplasmic tyrosine kinase domain. The extracellular portion of the protein interacts with fibroblast growth factors, setting in motion a cascade of downstream signals, ultimately influencing mitogenesis and differentiation. This particular family member binds both acidic and basic fibroblast growth factors and is involved in limb induction [11].

CONCLUSION:
Therefore, the results of work allow making a conclusion that genetic polymorphisms rs2252673, rs12444979, rs6732220, rs7538038, rs2013573, rs2288696 and rs10980926 are associated with the development of genital endometriosis. Combination of three genetic variants rs2013573 with rs2288696 and rs10980926 (OR=0.41) is protective factor of genital endometriosis, and combination genetic markers rs2252673 with rs12444979 with rs6732220 and rs7538038 to genital endometriosis (OR=4.81) is risk factor for genital endometriosis in the women of Russia Central Region.

REFERENCES: