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Proceeding Paper

Associations of Candidate Genes with the Level of Sex Hormones in Endometriosis †

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Abstract: Endometriosis is a chronic hormone-dependent inflammatory disease determined by the presence of foci of endometrial tissue outside the uterine cavity. Genetic factors occupy a leading position in the etiopathogenesis of this disease. The aim of the study was to explore the associations of polymorphism of sex hormone genes with the hormonal profile of patients with endometriosis. The study group included 103 patients with endometriosis, who were examined for levels of sex hormones (follicle-stimulating hormone, luteinizing hormone, prolactin, estradiol, progesterone, testosterone and dehydroepiandrosterone). The genotyping of 9 single nucleotide polymorphisms (SNP) of GWAS-significant sex hormone genes was carried out. The associations of SNPs with the level of sex hormones in patients with endometriosis were investigated by linear regression. Among patients with endometriosis, the serum estradiol level is associated with polymorphic loci rs148982377 *ZNF789* ($\beta = -0.488$–-0.445, $p_{perm} \leq 0.050$) and rs34670419 *ZKSCAN5* ($\beta = -0.544$–-0.449, $p_{perm} \leq 0.050$), luteinizing hormone—rs117585797 *ANO2* ($\beta = 0.618$–0.709, $p_{perm} \leq 0.050$), progesterone—rs117145500 *CHD9* ($\beta = 0.365$–0.429, $p_{perm} < 0.050$), prolactin—rs1641549 *TP53* ($\beta = -0.306$–-0.218, $p_{perm} < 0.050$), testosterone—rs148982377 *ZNF789* and rs34670419 *ZKSCAN5* ($\beta = 0.492$, $p_{perm} = 0.050$). Associations of candidate gene polymorphism with the level of sex hormones in patients with endometriosis have been established.

Keywords: endometriosis; sex hormones; polymorphism; associations

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1. Introduction

Endometriosis is a gynecological disease characterized by the growth of endometrial-like tissues inside and outside the pelvic cavity [1]. The prevalence of this disease ranges from 2% to 11% among women without clinically significant symptoms, from 5% to 50% among women suffering from infertility and from 5% to 21% among women with chronic pelvic pain [2]. Endometriosis is manifested by various symptoms, including dysmenorrhea, chronic pelvic pain, infertility, dyspareunia, etc. [3] and there is a wide range of socio-economic consequences for women suffering from this disease, their families, as well as society as a whole [4].

The endometriosis pathogenesis persists very "mysterious" and largely unknown [5]. The etiopathogenesis of this disease includes many hormonal, inflammatory, genetic, immunological factors, as well as environmental factors [3,6]. The development of endometriosis is associated with hereditary factors [5,7–9]. The contribution of hereditary factors according to several studies (a twin-based study, genome-wide study (GWAS), the SNP heritability) varies widely from 5 to 47% [5,7,10,11].

Sex hormones are important in the pathogenesis of endometriosis (follicle-stimulating hormone, progesterone,estradiol, etc.) [4,12,13]. In the literature, there are data ob-

tained in GWAS studies on genetic markers of the level of sex hormones [15–18]. Sex hormone genes are associated with the risk of developing endometriosis (*FSHB,LHCGR,ESR1,SYNE1,* etc.) [5,14,19,20]. However, there is insufficient data on this issue in the literature and further research on this topic is necessary.

The aim of the study: to explore the associations of polymorphism of sex hormone genes with the hormonal profile of patients with endometriosis.

2. Methods

2.1. Study Subjects

The DNA samples of 103 patients with endometriosis of Russian nationality living in the Belgorod region, born in the Central Chernozem region of Russia, served as the material for the study [21]. The average age of patients with endometriosis was 34.19 ± 6.42 years. The study examined the level of the following sex hormones in patients with endometriosis: follicle-stimulating hormone, luteinizing hormone, prolactin, estradiol, progesterone, testosterone and dehydroepiandrosterone.

2.2. Genetic Methods

In the studied sample of women, genotyping of 9 GWAS-significant polymorphic loci of sex hormone genes (rs148982377, rs34670419, rs11031002, rs11031005, rs112295236, rs117585797, rs117145500, rs727428, rs1641549) was performed, which are characterized by a pronounced regulatory potential (according to the HaploReg database [22]). Genotyping of the studied 9 polymorphic loci of sex hormone genes was carried out on the CFX-96 Real-Time System (Bio-Rad, Hercules, CA, USA) amplifier [23].

2.3. Statistical Methods

To evaluate the compliance of the empirical distribution of genotypes with the theoretically expected one at the Hardy-Weinberg equilibrium, the criterion χ^2 was used. The study of associations of polymorphic loci of sex hormone genes with a hormonal profile in patients with endometriosis was carried out in the PLINK program by linear regression [24]. Due to the fact that the distribution of the considered quantitative indicators characterizing the level of sex hormones differed from normal ($p < 0.05$), we used their transformed values in the calculations.

3. Results and Discussion

The distribution of all analyzed 9 molecular genetic markers included in the study corresponds to the Hardy-Weinberg equilibrium ($p > 0.05$), as well as the frequency of rare alley (MAF) > 5%. Correlations between the level of sex hormones in patients with endometriosis were studied (Table 1). Positive correlations were found between the level of follicle-stimulating and luteinizing hormones (R = 0.346, $p = 0.0004$). Positive correlations were also obtained between the level of luteinizing hormone and prolactin (R = 0.298, $p = 0.003$).

Table 1. Correlations between the level of sex hormones in patients with endometriosis.

	FSH	**LH**	**PROL**	**ESTR**	**PG**	**TEST**	**DHEAS**
FSH	1	0.0004	0.215	0.926	0.933	0.304	0.902
LH	0.346	1	0.003	0.643	0.093	0.224	0.821
PROL	0.126	0.298	1	0.152	0.698	0.370	0.643
ESTR	−0.009	−0.047	0.147	1	0.083	0.318	0.621
PG	−0.011	−0.0217	−0.050	0.222	1	0.810	0.369
TEST	−0.108	−0.128	0.094	0.106	0.032	1	0.568
DHEAS	−0.016	0.029	0.061	0.064	−0.152	0.075	1

Note: Spearman correlation coefficients are shown on the left, their significance levels are shown on the right; FSH—follicle stimulating hormone, LH—luteinizing hormone, PROL—prolactin, ESTR—estradiol, PG—progesterone, TEST—testosterone, DHEAS—dehydroepiandrosterone.

Associations of polymorphism of sex hormone genes with the level of sex hormones in patients with endometriosis have been established. Polymorphic loci rs148982377 and rs34670419, luteinizing hormone—rs117585797, progesterone—rs117145500, prolactin—rs1641549, testosterone—rs148982377 and rs34670419 are associated with the concentration of estradiol (Table 2). Markers of low levels of estradiol and prolactin in the serum of patients with endometriosis are alleles C rs148982377, T rs34670419 and T rs1641549, respectively, and markers of high levels of luteinizing hormone and progesterone are alleles A rs117585797 and C rs117145500, respectively (Table 2). High testosterone levels in women with endometriosis are marked by the haplotype TG rs148982377-rs34670419 (β = 0.492, p = 0.037, p_{perm} = 0.050).

Table 2. Associations of candidate genes with the level of sex hormones in patients with endometriosis.

Hormone	SNP (MAF)	n	Association	Model
			β, p (p_{perm})	
estradiol	rs148982377 (C)	100	β = −0.488, p = 0.035 (p_{perm} = 0.050)	allelic
estradiol	rs148982377 (C)	100	β = −0.445, p = 0.042 (p_{perm} = 0.050)	additive
estradiol	rs148982377 (C)	100	β = −0.445, p = 0.044 (p_{perm} = 0.050)	dominant
estradiol	rs34670419 (T)	100	β = −0.544, p = 0.026 (p_{perm} = 0.047)	allelic
estradiol	rs34670419 (T)	100	β = −0.449, p = 0.050 (p_{perm} = 0.049)	additive
estradiol	rs34670419 (T)	100	β = −0.449, p = 0.050 (p_{perm} = 0.050)	dominant
luteinizing	rs117585797 (A)	101	β = 0.709, p = 0.010 (p_{perm} = 0.050)	allelic
luteinizing	rs117585797 (A)	101	β = 0.618, p = 0.028 (p_{perm} = 0.030)	additive
luteinizing	rs117585797 (A)	101	β = 0.618, p = 0.028 (p_{perm} = 0.033)	dominant
progesterone	rs117145500 (C)	63	β = 0.366, p = 0.021 (p_{perm} = 0.044)	allelic
progesterone	rs117145500 (C)	63	β = 0.365, p = 0.028 (p_{perm} = 0.036)	additive
progesterone	rs117145500 (C)	63	β = 0.429, p = 0.030 (p_{perm} = 0.020)	dominant
prolactin	rs1641549 (T)	101	β = −0.218, p = 0.038 (p_{perm} = 0.032)	allelic
prolactin	rs1641549 (T)	101	β = −0.233, p = 0.022 (p_{perm} = 0.019)	additive
prolactin	rs1641549 (T)	101	β = −0.306, p = 0.020 (p_{perm} = 0.022)	dominant

Note: The results were obtained by linear regression analysis, taking into account the correction for covariates; MAF—minor allele, β—linear regression coefficient (change of the transformed hormone level indicator to a minor allele), p—significance level, p_{perm}—the significance level of the models after the permutation test.

According to the data previously obtained by GWAS, the polymorphisms rs11031002 and rs11031005 of the *FSHB* gene analyzed in this study are associated with various phenotypes characterizing a woman's reproductive system both in normal and various diseases. The polymorphic locus rs11031002 is associated with the level of luteinizing hormone [17], the level of serum protein such as CGA/FSHB [25], syndrome of polycystic ovary [26], bone mineral density [27].

The molecular genetic marker rs11031005 links with endometriosis/migraine [14], the level of follicle-stimulating hormone [17], the concentration of total and bioavailable testosterone [18], the age of menarche [28] and menopause [29], syndrome of polycystic ovary [30]. The above data marked expressed "pleiotropic" effects of *FSHB* gene SNPs. The literature data show the features of the hormonal status of endometriosis women such as reduced production (frequencies, amplitudes) of gonadotropin-releasing hormone and luteinizing hormone, which leads to a decrease in the level of luteinizing hormone, an

increase in follicle-stimulating hormone, a decrease in the ratio of luteinizing hormone/follicle-stimulating hormone, an increase of globulin binding sex hormones concentration, a decrease of testosterone (serum and follicular), pronounced changes of estradiol concentration and estradiol/testosterone ratio, a change in the expression of aromatase, etc.

4. Conclusions

Associations of candidate gene polymorphism with the level of sex hormones in patients with endometriosis have been established.

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Conflicts of Interest: The authors declare no conflict of interest.

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