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ESR1, FSH-receptor, and GSTM1, Polymorphisms in Infertile Greek Women with Advanced Endometriosis – A Pilot Study

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Abstract

Background: Endometriosis is an estrogen-dependent inflammatory condition, which is characterized by the presence of endometrium-like tissue in sites outside the uterine cavity. Although the etiology and pathogenesis of endometriosis remains largely unknown, disease susceptibility seems to depend on a complex interaction between environmental hormonal and genetic factors. It has been shown that genetic variations in Estrogen and FSH Receptors (ESR1 and FSHR respectively) may contribute to the pathogenesis of endometriosis. Additionally because of the detoxifying properties of the GST family, loss of their function, or altered expression due to the presence of a polymorphism, may facilitate development of endometriosis. **Materials and Methods:** The study included twenty consecutive infertile Greek women who were operated laparoscopically in our Gynecological Endoscopy Unit for advanced endometriosis and 48 parous women as controls with a history of at least one successful pregnancy, no history of spontaneous abortion, and no known history of endometriosis. Real time PCR was applied in all samples to detect the distribution of Estrogen Receptor (ESR1), Follicle Stimulating Hormone Receptor (FSHR) and Glutathione S-transferase Mu 1 (GSTM1) polymorphisms. **Results:** In blood samples, the presence of the Pvull polymorphism in both alleles was found in only 6.7% of patients with endometriosis, compared with 35.4% of controls (p=0.033). The wild type (WT) form of the FSHR was found less commonly in patients with endometriosis compared with controls (13.3% *vs.* 37.5%, p=0.081). Similarly, detection of this polymorphism in both alleles of the FSHR was more common in endometriosis (40% *vs.* 18.8% p=0.09). The GSTM1 gene polymorphism was detected in the majority of controls (83.3%). It was interesting to note that certain combinations of haplotypes (ESR1/FSHR/GSTM1) were absent from the blood samples of patients with endometriosis. **Conclusions:** An establishment of a genetic profile associated with endometriosis may be possible if a larger number of samples are studied, indicating possible mechanisms associated with the pathophysiology of the disease.

Introduction

Endometriosis is an estrogen-dependent inflammatory condition, which is characterized by the presence of endometrium-like tissue in sites outside the uterine cavity [1]. Although the etiology and pathogenesis of endometriosis remains largely unknown, disease susceptibility seems to depend on a complex interaction between environmental hormonal and genetic factors [2,3]. Epidemiological studies have shown that presence of a genetic component may increase the risk of endometriosis in near relatives [4].

Estrogen plays an important role in endometriosis by promoting growth of endometriotic tissue, and by sustaining cell survival and differentiation [5,6]. There are two intracellular receptors, ESR-α and β, encoded by the *ESR1* and *ESR2* genes, respectively, and both are expressed in normal endometrium and in endometriotic lesions [7]. It has been shown that genetic variations in ERs may contribute to the pathogenesis of endometriosis [8,9].

Circulating estrogens synergize with FSH to increase the number of FSHR in granulosa cells, and in turn, FSH stimulates granulosa cells to produce estrogens [10]. Studies have revealed the presence of multipotent FSHR-expressing granulosa cells with prolonged life-span, supporting the hypothesis that these multipotent cells and the FSH receptors may play an important role in the pathogenesis of ovarian endometriosis [11]. Variations and gene polymorphisms of the FSH receptor (FSHR) have also been associated with endometriosis [12,13].

Glutathione-S-transferases (GSTs) are key enzymes of cell cycle phase II, during which glutathione is conjugated to different potentially genotoxic compounds [14]. Several

studies have addressed the possible correlation between endometriosis and GST polymorphisms [15,16]. In serum, peritoneal fluid and tissues of patients with endometriosis, markers of oxidative stress- against which Gstm1 plays an important role- have been found elevated [17,18]. Because of the detoxifying properties of the GST family, loss of their function, or altered expression due to the presence of a polymorphism, may facilitate development of endometriosis [19].

The aim of the present study is to establish a possible association between a particular genetic profile of women with endometriosis and their clinical characteristics, and also to provide insights on the molecular and cellular mechanisms associated with this disease.

Materials and Methods

Subjects

This prospective study was conducted in the 1st Department of Obstetrics and Gynecology of the National and Kapodistrian Univesity of Athens, Greece. We included 20 consecutive infertile women of Caucasian origin who were operated laparoscopically in our Gynecological Endoscopy Unit for advanced endometriosis. In all cases disease was confirmed histologically, and tissue samples from ovarian endometriomas and eutopic endometrium were collected. Written informed consent was read and signed by all participants. The study has been approved by the Review Board of our Institution. In 15/20 of these patients, we collected also preoperative blood samples. As controls, we used peripheral blood collected from 48 parous women with a history of at least one successful pregnancy, no history of spontaneous abortion, and no known history of endometriosis.

GSTM1 genotyping

DNA samples were subjected to polymerase chain reaction amplification to determine the presence of GSTM1. The obtained PCR product from each patient was visualized in an ethidium bromide stained 3% agarose gel. The presence of GSTM1 allele was shown as a fragment of 219bp, while if the study subject is null for the gene, no PCR product is present.

Detection of PvuII and Asn/Ser 680 Polymorphisms

Patients were genotyped for PvuII (c.454-397T>C rs2234693) and Asn/Ser680 (N680S rs6166) polymorphisms. Both polymorphisms were performed with Real-Time PCR, using the Light Cycler 480 II (Roche Diagnostics, Germany).

Statistics

Chi-square goodness-of-fit test and binomial test were used for comparing ratios among genotypes within the same group and the same gene. To compare the distribution of genotypes between case and control samples we used binary Logistic Regression.

Results

Twenty infertile women with advanced endometriosis were studied for the presence of PvuII, Ser680Asn and *GSTM1* polymorphisms in tissue sampled from eutopic endometrium and ovarian endometriomas. In 15 of these patients blood samples was also possible to obtain and the above polymorphisms were also analyzed, and compared with those obtained from 48 controls.

In blood samples, if we consider the wild type genotype of ESR1 gene as a reference genotype there is no statistically significant differences between controls and endometriosis cases for both heterozygous and homozygous for the polymorphism alleles (p=0.179 and p=0.297 respectively).

On the contrary for the FSHR gene if the wild type genotype is considered as a reference there is a statistically significant difference for the homozygous alleles between controls and endometriotic cases (p=0.049). No statistically significant difference was found between cases and controls for the heterozygous genotype (p=0.203).

The *GSTM1* gene polymorphism was detected in the majority of controls (83.3%). Its detection in patients with endometriosis was also common but at a lower rate (66.7% p=0.429) (Table 1).

SNP	Genotype	Controls (N=48)	Cases (N=15)	Odds Ratio (95%CI)	p-value
ESR1	WT	29,2% (14/48)	26,7% (4/15)	Reference genotype	
	MT	35,4% (17/48)	6,7% (1/15)	0.206 (0.021 – 2.059)	0.179
	HT	35,4% (17/48)	66,7% (10/15)	2.059 (0.529 – 8.008)	0.297
FSHR	WT	37,5% (18/48)	13,3% (2/15)	Reference genotype	
	MT	18,8% (9/48)	40,0% (6/15)	6.00 (1.003 – 35.908)	**0.049**
	HT	43,8% (21/48)	46,7% (7/15)	3.00 (0.552 – 16.305)	0.203
GSTM1	Yes	83,3% (40/48)	66,7% (10/15)	1.666 (0.470 – 5.906)	0.429
	No	16,7% (8/48)	33,3% (5/15)		

Table1: Comparison of different genotypes of ESR1, FSHR and GSTM1 polymorphisms between controls and endometriotic cases.

Comparison of different possible combinations of genotypes (ESR1/FSHR/GSTM1) between the two groups, showed that certain combinations were absent from the blood samples of patients with endometriosis: TT, Asn/Asn, null, TC/CC, Asn/Asn, null, and TT, Asn/Asn, GSTM1 (Table 2).

ESR1	FSHR	GSTM1	CONTROLS	%	CASES	%
TT	Asn	NULL	2/48	4.1	0/15	0
T/C,C/C	Asn/Asn	NULL	3/48	6.25	0/15	0
TT	Asn/Ser, Ser/Ser	NULL	2/48	4.1	1/15	6.6
T/C,C/C	Asn/Ser, Ser/Ser	NULL	2/48	4.1	3/15	20
TT	Asn	GSTM1	3/48	6.25	0/15	0
T/C,C/C	Asn	GSTM1	9/48	18.75	3/15	20
TT	Asn/Ser, Ser/Ser	GSTM1	7/48	14.5	2/15	13.3
T/C,C/C	Asn/Ser, Ser/Ser	GSTM1	20/48	41.6	6/15	40

Table 2: Genetic profile in blood of controls and patients with endometriosis.

In tissue samples, detection of PvuII SNP did not reveal significant differences between eutopic, and ectopic tissues. The *ESR1* polymorphism was detected in 80% *vs.* 70% of cases (p=0.733 for the mutant allele and p=0.457 for the heterozygous alleles).

Similarly, detection of the *FSHR* polymorphism was identical in both tissue types (80%, p=1).

The *GSTM1* polymorphism was found at similar rates in both types of tissue: 75% *vs.* 83% for eutopic and ectopic endometrium, respectively.

The establishment of a genetic profile in tissue showed that several genotypes were also not represented in samples from patients with endometriosis. Simultaneous detection of wild type alleles of both ESR1 and FSHR genes with concurrent absence of the *GSTM1* polymorphism (TT, Asn/Asn, null) was not shown in any of the samples. The same applied for the simultaneous detection of wild type alleles of ESR1, FSHR and presence of the GSTM1 polymorphism (TT, Asn/Asn, GSTM1). All the above missing genotypes were identical to those that were absent from blood of patients with the disease (Table 3).

ESR1	FSHR	GSTM1	Eutopic endometrium	%	Ectopic endometrium	%
TT	Asn/Asn	NULL	0/20	0	0/20	0
T/C, C/C	Asn/Asn	NULL	0/20	0	2/20	10
T/C, C/C	Asn/Ser, Ser/Ser	NULL	1/20	5	0/20	0
TT	Asn/Ser, Ser/Ser	NULL	3/20	15	1/20	5
TT	Asn/Asn	GSTM1	0/20	0	0/20	0
T/C, C/C	Asn/Asn	GSTM1	4/20	20	2/20	10
TT	Asn/Ser, Ser/Ser	GSTM1	2/20	10	4/20	20
T/C, C/C	Asn/Ser, Ser/Ser	GSTM1	10/20	50	11/20	55

Table 3: Genetic profile in eutopic and ectopic endometrium of patients with endometriosis.

Discussion

Endometriosis seems to be an estrogen dependent disorder, as estrogen and its receptors have a key role in the pathogenesis of this disease, even though genetic defects, immunity and environmental and other hormonal factors may also contribute. Polymorphisms and estrogen receptor related genotypes may influence the sex-steroid system both at the receptor and the hormone synthesis level. Additionally, estrogen production plays an essential role in the evolution of endometriosis by enhancing survival of the endometriotic tissue and by mediating pelvic pain and infertility with prostaglandins and cytokines.

In our study we evaluated the possible association of the detection of one of three polymorphisms, or of a genetic polymorphic combination with presence of endometriosis. We also tested the hypothesis of whether certain SNPs or genotype combinations are present uniquely in endometriotic tissues or are tissue independent.

It appears that presence of the *ESR1* PvuII and *FSH* Asn680Ser polymorphisms does not differ between eutopic and ectopic endometrium. The same applies for the *GSTM1* polymorphism which is also highly detected in both tissues.

Rates of detection of all 3 polymorphisms were also very similar in the blood of cases with endometriosis, indicating that the polymorphic combinations apply to any type of sample taken from these patients.

It was interesting to note the significantly higher detection rates of the homozygous type of the FSHR polymorphism found in blood, in patients with endometriosis compared with controls (p=0.049).

The above observation may have significant biological consequences. A polymorphic gene encodes for a protein with an altered spatial conformation and consequently its interaction with the hormone is different. An altered interaction because of the presence of SNPs between estrogen and *ESR1*, may lead to an altered estrogen response which in turn may influence the ERα-TNF pathway. We would expect that the presence of a variant should act as an antagonist in the TNF pathway, but our results demonstrate that probably the presence of a single allelic variant does not alter the endometriotic burden. On the other hand, a polymorphic *FSHR* may have consequences on the activity of aromatase and subsequent production of estradiol. The question that remains is how SNPs of estrogen and FSH receptors may be associated with the development of endometriosis, as it is shown that they are highly represented.

Several studies have shown a positive correlation between endometriosis and *ESR1* polymorphisms, whereas others reported no significant association. Pasculin et al. showed no association between ESR1 PvuII polymorphisms and endometriosis [20]. On the contrary, Lamp et al. demonstrated a possible association between ESR1 polymorphisms and the disease in women with endometriosis but no associated infertility [21]. Wang et al have associated the *ESR1*rs3798573 polymorphism with risk of endometriosis and infertile endometriosis in Han Chinese women [22]. In our study the *ESR1* polymorphism in question was found less commonly in a homozygous condition in patients with endometriosis in comparison with controls, a finding of unclear clinical significance.

Infertility is multifactorial. It is well known that endometriosis affects ovarian function and implantation rates. It is possible that *ESR1* polymorphisms may be involved in ovarian responsiveness and endometrial receptivity. The *ESR1* rs2234693 (PvuII) polymorphism which has been found to be more prevalent in infertile women with premature ovarian aging, it was also predictive of an improved controlled ovarian stimulation. Both XbaI and PvuII SNPs are associated with differences of responses to ovarian stimulation, and probably have an indirect role in implantation rates. The presence of 3 or more polymorphic alleles was associated with significantly lower E2 levels on the day of hcg administration and a significantly lower rate of good quality embryos [23]. In our study the presence of *ESR1* polymorphism is independent of the presence of endometriosis and the homozygous genotype is poorly represented. The question that remains is whether women with endometriosis and carrying the PvuII polymorphism have poor response to ovarian stimulation.

Regarding the FSHR Asn680Ser polymorphism there are contradictory results. Schmitz et al found no association of the FSHR Asn680Ser polymorphism with endometriosis [24]. Wang HS et al. have shown that women with this polymorphism had a significantly lower risk for developing endometriosis [25]. Kerimoglu et al. found that when their endometriosis patients were divided into two groups according to disease severity, those with the SS (680 Ser/Ser) or AA (307 Ala/Ala) genotype were less likely to have stage 3 and 4 endometriosis compared to stage 1 and 2 (P=0.004, or 0.177, 95% CI 0.055-0.568 and P=0.040, or 0.240, 95% CI 0.061-0.938; respectively) [26]. In our study the presence of polymorphism seems to be associated with endometriosis.

Our previous study in infertile women has demonstrated that S/S, and A/A genotype of *FSHR* presented slightly higher FSH level and also gives less number of oocytes and less E2 level the day of hcg administration [27]. This picture is associated to a poor ovulation profile. It would be interesting to investigate the impact of the above genotypes in women with endometriosis when entering an IVF treatment.

The presence of polymorphisms in GST genes and their relation with the risk of developing different diseases has provoked the interest for studying its correlation with endometriosis. Fraze et al. detected a higher absence of *GSTM1* in women with endometriosis (61%), while the control group displayed a greater presence of the corresponding polymorphism, which suggested that GSTM1 is unrelated to the cell proliferation of endometriosis [28]. Lin et al. have found a significant association between *GSTM1* and endometriosis (72.1% null genotype in endometriosis versus 42.9% in controls) [29]. Hue et al. found no association between *GSTM1* polymorphisms and endometriosis [30]. Their study included 194 women with endometriosis confirmed by laparoscopy and 259 controls (laparoscopy and laparotomy without disease). In our study the higher absence of *GSTM1* gene in blood of women with endometriosis, may indicate a possible involvement of the detoxifying metabolic pathway in the pathophysiology of the disease.

Conclusions

In our study the simultaneous presence of PvuII, Asn680Ser polymorphisms and the absence of GSTM1 gene were more detected in women with endometriosis than in controls indicating that probably the above genetic profile is associated with the pathophysiology of the disease. Additionally endometriosis may be influenced by the specific interactions between the hormonal mechanisms of reproduction and by the altered expression of related proteins. Further studies including a larger number of patients are needed.

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