Catechol-*O*-Methyltransferase Polymorphism and Endometriosis

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Purpose: Catechol-*O*-methyltransferase (COMT) inactivates the estradiol metabolites, 2-hydroxy and 4-hydroxy catechols, which have been implicated in the pathogenesis of endometriosis. A COMT valine to methionine polymorphism (G-to-A) in exon 4 of the COMT gene is polymorphic in the human population, with 25% of Caucasians being homozygous for the low-activity allele (COMT-L) of the enzyme. In a case-control study we investigated whether this COMT polymorphism is associated with endometriosis.

Methods: Polymerase chain reaction was performed to analyze the COMT genotype among women with surgically and histologically confirmed endometriosis (study group; $n = 91$) and in women without evidence of endometriosis confirmed by laparoscopy or laparotomy (control group; $n = 92$).

Results: Allele frequencies for the low-activity allele (COMT-L) among women with endometriosis and controls were 0.50 and 0.50, respectively ($p = 0.999$; odds ratio = 1.0, 95% CI: 0.66–1.51).

Conclusions: Our results suggest that the valine to methionine polymorphism in exon 4 of the COMT gene is not associated with the risk of endometriosis compared to a surgical control population.

KEY WORDS: Endometriosis; COMT; polymorphism; catecholestrogens; Caucasian population.

INTRODUCTION

Immunologic, genetic, environmental, and hormonal factors contribute to the multifactorial etiology of endometriosis (1). The implantation hypothesis of Sampson is the most widely accepted etiologic theory of endometriosis (2). An increasing body of evidence suggests that altered local

immunologic reactions directed against physiologic retrograde menstruation products may support the attachment, growth, and persistence of ectopic endometrial cells (3). Epidemiologic data showed a familial tendency for endometriosis, and it is likely that heritable genetic factors strongly contribute to the development of endometriosis (4). Polymorphisms in genes encoding conjugating enzymes, such as glutathione *S*-transferase M1 (GSTM1) gene and *N*-acetyltransferase-2 (NAT-2) gene, and estrogen receptor (ER)- α gene polymorphisms have been found to be associated with endometriosis (5–8). Recently, the PP genotype of the PVUII polymorphism (PP, Pp, and pp) of the ER- α gene was shown to be associated less frequently with endometriosis (6). This PVUII polymorphism in the ER- α gene may influence

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estrogen receptor action as a modulator of the ligand leading to aberrant expression of ER in estrogen dependent diseases including endometriosis (6). In accordance, conjugating enzymes such as GSTM1 and NAT-2 enzymes usually function to inactivate carcinogenic and procarcinogenic compounds, which indicates a role of environmental toxins in the etiology of endometriosis (8).

Endometriosis affects up to 15% of women almost exclusively in the reproductive age (1). Only 2–4% of all women with endometriosis who require laparoscopy for endometriosis are postmenopausal (1,9). The majority of such postmenopausal women with endometriosis have received hormone replacement therapy. This epidemiologic data and laboratory evidence suggest that endometriosis is an estrogen-dependent disease (10). ER- α and progesterone receptor (PR) were found to be expressed in endometriotic tissues (11). Therefore, hormonal treatment with danazol, progestins, and gonadotropine-releasing hormone agonists (GnRHa) are prescribed for the treatment of endometriosis associated symptoms (1).

17 β -estradiol is metabolized via one of the two major pathways, namely 16 α -hydroxylation and formation of catecholestrogens namely the 2-hydroxy and 4-hydroxycatechols (2-HE and 4-HE) (12,13). Catechol-*O*-methyltransferase (COMT) is a ubiquitous enzyme of the estrogen metabolizing pathway that catalyzes *O*-methylation and subsequent inactivation of 2- and 4-hydroxycatechols (14). The level of COMT enzyme activity is genetically polymorphic in human tissues with a trimodal polymorphic distribution of low activity (COMT^{LL}), intermediate (COMT^{LH}), and high (COMT^{HH}) activity (14). Twenty-five percent of the Caucasian population carry the low-activity COMT genotype, which is caused by an evolutionarily recent G to A misense mutation that translates into a substitution of Met for Val at codon 158 (G-to-A) (GenBank accession No. Z26491). The low-activity allele (COMT-L) of the COMT polymorphism, which is associated with higher levels of catecholestrogens, was found more frequently in women with breast cancer (15) than in healthy controls particularly in women with menopausal symptoms (16). Catecholestrogens were suggested to play a role in the pathogenesis of endometriosis (17,18). To explore the association of COMT polymorphism and endometriosis, we investigated whether the low-activity COMT-L allele is increased among women with endometriosis.

PATIENTS AND METHODS

Patients

All women were Caucasians. The study cohort comprised women with surgically and histologically confirmed endometriosis (study Group: $n = 91$), who underwent surgery at our department. According to the revised American Fertility Score (rAFS) classification (19), all women were staged into two groups: Group I = rAFS Stage I/II = minimal/mild endometriosis; Group II = rAFS Stage III/IV = moderate/severe endometriosis. Study patients underwent diagnostic laparoscopy or laparotomy because of benign ovarian masses ($n = 38$), infertility ($n = 34$), chronic pelvic pain ($n = 18$), and miscellaneous indication ($n = 1$). We included a surgical control group of 92 Caucasian women with surgically confirmed absence of endometriosis, who were admitted for laparoscopy or laparotomy for infertility ($n = 38$), benign ovarian masses ($n = 35$), chronic pelvic pain ($n = 12$), and miscellaneous indications ($n = 7$). All women were recruited at the Division of Endocrinology and Assisted Reproduction in the University Hospital of Vienna. After ethics committee approval, written informed consent was obtained from all participating women before blood samples were taken.

Genotyping Methodology

Human leukocyte genomic DNA was extracted from whole peripheral blood samples using the QIAGEN System (QIAmp DNA Blood Midi Kit, Hilden, Germany). DNA was stored at 4°C until analyzed. Genomic DNA was analyzed for the presence of the G-to-A transition in codon 158 of the COMT gene by a polymerase chain reaction (PCR)-based RFLP assay. A 237-bp genomic fragment, including the part of exon 4 that contains the polymorphic site, was amplified by PCR using the forward primer TACTGTGGCTACTCAGCTGTGC (pos. 1827–1848), (20) and the reverse primer GTGAACGTGGTGTGAACACC 8 (pos. 2044–2063), (20). Amplification reactions were performed on a Perkin-Elmer GeneAmp template, 25 pmol of each primer, 250 μ M dNTPs, 1 $\times$ SuperTaq Buffer (ViennaLab, Austria), and 0.5 units SuperTaq DNA Polymerase (ViennaLab). The amplification profile was as follows: 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s; 35 cycles. To simplify the performance and to increase reproducibility of PCR, PCR-mastermixes containing primers, dNTPs, and buffer were prepared and used in all amplification reactions. In addition,

Fig. 1. PCR-based RFLP analysis of the COMT (codon 158) polymorphism. Lane 1 represents a heterozygous sample (COMT^{HL}), where one allele contains a G and the second one an A at pos. 1947, Lane 2 a homozygous sample (COMT^{LL}), where both alleles contain an A at pos. 1947 and Lane 3 another homozygous sample (COMT^{HH}), where both alleles contain a G at pos. 1947 (the 18 bp restriction fragments from Lanes 1 and 2 run off the gel during electrophoresis).

tubes containing all PCR components and distilled water instead of DNA served as negative controls to check for the presence of DNA that may have been carried over from prior reactions. A total of 5 μ L of each PCR product were run on agarose gels to ensure that the expected 237 bp product was generated. The remaining 45 μ L were purified by combined ammoniumacetate/ethanol precipitation and digested overnight with 10U *Nla*III (New England Biolabs, MA) at 37°C. The products of the restriction digest were separated on agarose gels (4% SB Fine Gel Agarose) (Severn Biotech Ltd., UK) and visualized by SYBR Green I (Molecular Probes Inc., OR). Restriction fragments of 27, 42, and 54 bp were present in every digested sample (Fig. 1). In the presence of a G at position 1947 (20), an additional 114-bp fragment was present, which was cut by *Nla*III into 96- and 18-bp fragments when position 1947 contained an A (Fig. 1).

Statistical Analysis

Allele frequencies and genotype frequencies were compared between the study group and the control group using the multiple Pearson chi-square test of independence with Yates' continuity correction. The

odds ratio (OR) approximates the risk ratio between the occurrence of a single allele or a genotype in the endometriosis group. Statistical analyses were performed by the use of SPSS (SPSS Inc., Chicago, IL 60606) for Windows 10.0.1 (1999). All *p* values were two-tailed, and 95% confidence intervals (CI) were calculated. The significance level was set to 0.05.

RESULTS

The median age of women with endometriosis was 34 years (range: 22–60) and of controls 35 years (range 18–76). Fifteen women had Stage I, 20 had Stage II, 37 had Stage III, and 19 had Stage IV of rAFS-score endometriosis. We divided women with endometriosis into two groups: Group I (rAFS I/rAFS II = minimal/mild endometriosis): *n* = 35; Group II (rAFS III/rAFS IV): *n* = 56.

All women were genotyped for the presence of the high-activity (COMT-H) and the low-activity (COMT-L) alleles. The distribution of COMT^{HH} (high activity), COMT^{LH} (intermediate activity), and COMT^{LL} (low activity) genotypes was 25.3, 50.5, and 24.2% and 25.0, 51.1, and 23.9% in the study group and in the controls, respectively (Table I). Twenty-two (24.2%) women in the study group and 22 (23.9%) women in the control group were shown to be homozygous for the COMT-L allele. The distribution of allele frequencies was not statistically different between the study group and the control group regarding the low-activity COMT-L allele (COMT-L vs. COMT-H; *p* = 0.999; OR = 1.00, 95% CI: 0.66–1.51; Table I).

Table I. Genotype and Allele Frequencies of COMT Polymorphism (Val158Met) Among Women with Endometriosis and Controls

	Women with EM ^a (<i>n</i> = 91)	Controls (<i>n</i> = 92)	OR (95% CI)	<i>p</i> value ^b
Genotypes				
COMT ^{HH}	23 (25.3%)	23 (25.0%)	1.02 (0.52–1.98)	ns ^c
COMT ^{LH}	46 (50.5%)	47 (51.1%)	0.98 (0.55–1.75)	ns
COMT ^{LL}	22 (24.2%)	22 (23.9%)	1.01 (0.52–2.00)	ns
Alleles				
COMT-H ^d	92 (50.5%)	93 (50.5%)		
COMT-L ^e	90 (49.5%)	91 (49.5%)	1.00 (0.66–1.51)	ns

^a EM = Endometriosis.

^b Chi-square test.

^c ns = not significant.

^d High-activity allele.

^e Low-activity allele.

Table II. Allele Frequencies of COMT Polymorphism (Val158Met) in Women with Minimal/Mild and Moderate/Severe Endometriosis (Group I: $n = 70$, and Group II: $n = 112$) and Controls ($n = 184$)

	COMT-H ^a ($n = 91$)	COMT-L ^b ($n = 92$)	OR (95% CI)	p value ^c
Controls	93 (50.5%)	91 (49.5%)	—	—
Group I (rAFS I/II)	35 (50.0%)	35 (50.0%)	—	ns ^d
Group II (rAFS III/IV)	57 (50.9%)	55 (49.1%)	—	ns

^a High-activity allele.

^b Low-activity allele.

^c Two-sided chi-square test comparing the frequency of the COMT-L allele of controls to Group I and Group II, respectively.

^d ns = not significant.

Statistical analysis of each endometriosis group (Groups I and II) showed that the occurrence of the COMT^{LH} or the COMT^{LL} genotype was not significantly different in women with minimal/mild endometriosis (Groups I: $n = 35$ (19.2%) vs. controls: $n = 92$ (50.3%); $p = 0.934$; OR = 1.0, 95% CI: 0.4–2.5) and in women with moderate/severe endometriosis (Group II: $n = 56$ (30.6%) vs. controls: $n = 92$ (50.3%); $p = 1.000$; OR = 1.0, 95% CI: 0.5–2.2) compared to controls. Furthermore there were no significant differences in frequencies of the COMT-L allele in women with minimal/mild endometriosis ($n = 70$ (19.1%)) as well in women with moderate/severe endometriosis ($n = 112$ (30.6%)) compared to controls (Table II).

DISCUSSION

In this study we investigated an association between the COMT valine to methionine polymorphism and the occurrence of endometriosis. Our theory to test the COMT gene as a candidate gene for endometriosis was supported by the evidence that the COMT pathway is one of the major degrading pathways of 17 β -estradiol and that its metabolites, namely catecholestrogens, are involved in the pathogenesis of endometriosis (12,13,17,18).

Increasing evidence indicates that several enzymes involved in estrogen synthesis and metabolism play a major role in the pathogenesis of endometriosis including the Cyp450 enzymes CYP1A1, and CYP19 (aromatase), and 17 β -hydroxysteroid dehydrogenase Type 2 (17HSD Type 2) (17,21). CYP1A1 is among the major enzymes participating in estrogen hydroxylation (17,22). CYP1A1 was found to be strikingly

higher in endometriotic tissues than in the eutopic endometrium (17). CYP1A1 is promoting the development and growth of endometriosis by either activating procarcinogens or inducing the formation of catecholestrogens or both (17). In accordance, CYP19, which catalyzes the formation of estrogens from C19 steroids in a number of tissues such as the ovary and the placenta, is significantly elevated in endometriotic cells (21). The expression of 17 β -HSD Type 2, which usually lowers estrogen, is absent from endometriotic glandular cells (21,23). The aberrant expression of CYP1A1, CYP19, and 17 β -HSD Type 2 in endometriotic tissue give rise to elevated levels of 17 β -estradiol by enhancing its production and diminishing its metabolism. In fact, higher 17 β -estradiol levels have been detected in the peritoneal fluid of women with endometriosis than in healthy controls (24).

O-methylation mediated by COMT is an important mechanism for inactivating catecholestrogens, which display selective estrogen activity in the mammary gland, bone, and in uterine tissues of humans, rabbits, and mice (14,25–28). In particular, 4-hydroxylation of estrogens was shown to be common in extrahepatic tissues including the pituitary, the myometrium, and myomal tissues (29). Moreover, catecholestrogens were shown to stimulate prostaglandin synthesis in the uterus (26,28). Prostaglandins were considered to exhibit important uterine functions and play a role in the pathogenesis of endometriosis. In the view of the proposed role of catecholestrogens in endometriosis and the higher tissue concentrations of catecholestrogens in carriers of the low-activity allele (COMT-L) (14), we examined the possible relationship between occurrence of the low-activity COMT genotype and endometriosis in a Caucasian population.

We investigated a common COMT valine to methionine polymorphism (G-to-A) in exon 4 of the COMT gene in women with endometriosis. We could not find an increased frequency of the low-activity COMT genotype in women with endometriosis. This is the first report of a valine to methionine polymorphism in exon 4 of the COMT gene in Caucasian women with endometriosis. The genotypes (COMT^{HH}, COMT^{LH}, and COMT^{LL}) of the COMT polymorphism in our surgical controls and were comparable to genotype frequencies reported in other control populations (14–16). The nonassociation of this COMT (VAL158MET) polymorphism in the COMT gene and endometriosis in a Caucasian population does not rule out the association of other

genes coding for enzymes in estrogen metabolism since it is evident that endometriosis is a polygenic disease. For example, the combination of the GSTM1 null genotype and the CYP1A1 MspI polymorphism was associated with a small increased risk of endometriosis (30).

In conclusion, in this study the valine to methionine COMT polymorphism (G-to-A) in exon 4 of the COMT gene does not show an association with endometriosis in a Caucasian population. This data indicates that the COMT gene is not a candidate gene for endometriosis.

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