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Association between *CYP19A1*, *GSTM1*, *GSTT1*, and *GSTP1* genetic polymorphisms and the development of endometriosis in a Chinese population

Y. Tuo*, J.Y. He*, W.J. Yan* and J. Yang

Renmin Hospital of Wuhan University, Reproductive Medicine Center, Wuhan, Hubei, China

*These authors contributed equally to this study.
Corresponding author: J. Yang
E-mail: tuoya_1990@163.com

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ABSTRACT. Endometriosis is a common, complicated, and highly heterogeneous endocrine disease. Many genetic factors could affect the development of endometriosis. We performed a case-control study to evaluate the association between polymorphisms in *CYP19A1* rs2899470, *GSTM1*, *GSTT1*, and *GSTP1* rs1695 and the development of endometriosis in a Chinese population. Between March 2014 and October 2015, 262 endometriosis patients and 275 control subjects were recruited from the Inner Mongolia Medical University. Genotyping was conducted using polymerase chain reaction-coupled with restriction fragment length polymorphism. Individuals carrying the TT genotype of *CYP19A1* rs2899470 expressed a higher risk of endometriosis than those carrying the GG genotype, and the adjusted ORs (95%CI) was

2.33 (1.27-4.33). Moreover, those with the TG + TT genotype were correlated with an elevated risk of endometriosis, compared to those with the GG genotype (OR = 1.48, 95%CI = 1.03-2.13). However, *GSTM1*, *GSTT1*, and *GSTP1* rs1695 polymorphisms did not affect the pathogenesis of endometriosis. In conclusion, our results suggested that *CYP19A1* rs2899470 polymorphism is associated with risk for endometriosis in the Chinese population.

Key words: *CYP19A1*; *GSTM1*; *GSTT1*; *GSTP1*; Polymorphism; Endometriosis

INTRODUCTION

Endometriosis is a common gynecological disease with a 20% morbidity rate, and it impacts both a women's physical and mental well being (McLeod and Retzloff, 2010; Parazzini et al., 2016). The pathogenesis of endometriosis is not well understood, and the development of this disease is affected by various genetic and environmental factors (McLeod and Retzloff, 2010; Parazzini et al., 2016). It has been reported that mutations in genes related to hormone receptor, matrix metalloproteinase, inflammatory factor and angiotensin I-converting enzyme (Lamp et al., 2010; Abutorabi et al., 2015; Kerimoglu et al., 2015; Yang et al., 2016) may contribute to disease onset.

The cytochrome P450 19A1 (*CYP19A1*) gene encodes the human estrogen synthase, also known as aromatase, which belongs to the cytochrome P450 (CYP450) family of proteins. CYP450 is a member of the CYP superfamily, and its gene is located on chromosome 15q22 to q24. Two studies have previously reported that *CYP19A1* polymorphism contributes to the pathogenesis of endometriosis (Szczepańska et al., 2013; Wang et al., 2014). It has been shown that glutathione S-transferases (GSTs) are involved in estrogen metabolism, and play an important role in endometriosis development (Kubiszeski et al., 2015; Henidi et al., 2015); polymorphisms in GST-encoding genes could influence the expression and function of CYP450. We conducted a case-control study to evaluate the association between *CYP19A1* rs2899470, *GSTM1*, *GSTT1*, and *GSTP1* rs1695 polymorphisms and the development of endometriosis in a Chinese population.

MATERIAL AND METHODS

Subjects

Between March 2014 and October 2015, 262 endometriosis patients were recruited from the Inner Mongolia Medical University. Diagnosis of endometriosis was confirmed by pathological examination.

At the same time, 275 control subjects were also enrolled into our study. The inclusion criteria were as follows: normal menstrual cycles and basic reproductive hormone levels. Individuals positive for diabetes, dysfunctional uterine bleeding, and other endocrine diseases were excluded from our study.

Demographic and clinical data of endometriosis patients and controls were obtained from medical records or self-designed questionnaires, which included information such as age, body mass index, as well as menarche age, gravidity and parity. Written informed consent was obtained from all study subjects prior to their enrollment. Our study protocol was in line with the institutional ethics requirements of the Affiliated Hospital of Inner Mongolia Medical University.

DNA extraction and genotyping

Blood samples were collected from all subjects into 5% EDTA-containing tubes before any treatments were performed. Samples were kept at -20°C, and genomic DNA was extracted using the Tiangen DNA Blood Min Kit (Tiangen Biotech, Beijing, China), according to the manufacturer's instructions.

Genotyping of *CYP19A1* rs2899470, *GSTM1*, *GSTT1*, and *GSTP1* rs1695 was conducted by polymerase chain reaction-coupled with restriction fragment length polymorphism (PCR-RFLP). The primer sequences for *CYP19A1* rs2899470, *GSTM1*, *GSTT1* and *GSTP1* rs1695 were as follows: the *CYP19A1* rs2899470 forward and reverse primers were 5'-CAGGTAGCTCTGTGGAATAGCC-3' and 5'-GCAGAAAATTAATGAGGACCACAG-3', respectively; the forward and reverse primers for *GSTM1* were 5'-GAACTCCCTGAAAAGCTAA G-3' and 5'-GTTGGGCTCAAATATACGGTGG-3', respectively; the forward and reverse primers for *GSTT1* were 5'-TTCCTTACTGGTCCTCACATCTC-3' and 5'-TCACCGGATCAT GGCCAGCA-3', respectively; the forward and reverse primers for *GSTP1* rs1695 were 5'-GTAGTTTGCCCAAGGTCAAG-3' and 5'-GAAGAGCCAAGGACAGGTAC-3', respectively. The β-globulin gene was used as control; the forward and reverse primers were 5'-CAACTTCATCCACGTTCACC-3' and 5'-GAAGAGCCAAGGACAGGTAC-3', respectively.

The amplification conditions for *CYP19A1* rs2899470 were as follows: initial denaturation at 94°C for 10 min; 34 cycles of 94°C for 30 s, 50°C for 60 s, and 72°C for 60 s; final extension at 72°C for 10 min. The amplification conditions for *GSTM1*, *GSTT1* and *GSTP1* rs1695 were: 95°C for 5 min; 34 cycles of 94°C for 60 s, 60°C for 60 s, and 72°C for 60 s; final extension at 72°C for 7 min. DNA fragments were visualized on a 4% agarose gel.

Statistical analysis

Categorical variables are reported as percentages of continuous variables in the form of means ± SD. Demographic and clinical characteristics between the endometriosis patients and controls were compared using Student *t*-tests. Genotype frequencies in the two study groups were analyzed by chi-square (χ^2) tests. We also assessed whether the gene frequencies of *CYP19A1* rs2899470, *GSTM1*, *GSTT1*, and *GSTP1* rs1695 were in accordance with Hardy-Weinberg equilibrium via χ^2 tests. Multiple logistic regression analysis was used to estimate the association between genetic variations in *CYP19A1* rs2899470, *GSTM1*, *GSTT1*, and *GSTP1* rs1695 and the risk of endometriosis. The results are reported as odds ratios (ORs) with 95% confidence intervals (CIs). The ORs were adjusted by potential confounding factors. All statistical analyses were performed using the SPSS version 16.0 software (SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

RESULTS

The demographic and clinical variables of investigated subjects are shown in Table 1. There was no significant differences in age (t = 1.38, P = 0.08), menarche age (t = 0.60, P = 0.28), gravidity (t = 0.56, P = 0.29), and parity (t = 0.74, P = 0.23) between endometriosis patients and controls.

Table 1. Demographic and clinical variables of subjects.

Variables	Patients (N = 262)	Controls (N = 275)	t value	P value
Age (years)	25.85 ± 2.17	26.12 ± 2.36	1.38	0.08
BMI (kg/m^2)	25.72 ± 3.51	22.64 ± 3.50	10.18	<0.001
Menarche age (years)	13.73 ± 1.56	13.81 ± 1.54	0.60	0.28
Gravidity	1.65 ± 1.25	1.59 ± 1.23	0.56	0.29
Parity	0.77 ± 0.62	0.73 ± 0.63	0.74	0.23

BMI = body mass index.

The genotype distributions of *CYP19A1* rs2899470, *GSTM1*, *GSTT1*, and *GSTP1* rs1695 are shown in Table 2. As demonstrated by χ^2 tests, a significant difference was observed in the genotype frequencies of *CYP19A1* rs2899470 between the endometriosis patients and controls (χ^2 = 8.89, P = 0.01). However, no significant difference was found in the genotype distributions of *GSTM1* (χ^2 = 1.29, P = 0.26), *GSTT1* (χ^2 = 1.26, P = 0.26), and *GSTP1* (χ^2 = 2.18, P = 0.34).

Table 2. Genotype frequencies of *CYP19A1* rs2899470, *GSTM1*, *GSTT1I,* and *GSTP1* rs1695.

Genotypes	Patients (N = 262)	%	Controls (N = 275)	%	χ^2 test	P value	χ^2 for HWE in patients	P value	χ^2 for HWE in controls	P value
*CYP19A1*rs2899470										
GG	90	34.35	120	43.64						
TG	130	49.62	131	47.64						
TT	42	16.03	24	8.73	8.89	0.01	0.19	0.66	1.98	0.16
GSTM1										
Present	150	57.25	144	52.36						
Null	112	42.75	131	47.64	1.29	0.26				
GSTT1										
Present	192	73.28	213	77.45						
Null	70	26.72	62	22.55	1.26	0.26				
*GSTP1*rs1695										
AA	112	42.75	124	45.09						
AG	118	45.04	128	46.55						
GG	32	12.21	23	8.36	2.18	0.34	0.01	0.91	1.59	0.21

The association between *CYP19A1* rs2899470, *GSTM1*, *GSTT1*, and *GSTP1* rs1695 polymorphisms and the risk of endometriosis are listed in Table 3. Individuals carrying the TT genotype of *CYP19A1* rs2899470 expressed a higher risk of endometriosis than those carrying the GG genotype, and the adjusted OR (95%CI) was 2.33 (1.27-4.33). Moreover, those with the TG + TT genotype were correlated with an elevated risk of endometriosis, compared to those with the GG genotype (OR = 1.48, 95%CI = 1.03-2.13). However, we did not find any association between polymorphisms in *GSTM1*, *GSTT1*, and *GSTP1* rs1695 and risk of endometriosis.

Table 3. Association between *CYP19A1* rs2899470, *GSTM1*, *GSTT1I*, and *GSTP1* rs1695polymorphisms and risk of endometriosis.

Genotypes	Patients (N = 262)	%	Controls (N = 275)	%	Adjusted OR (95%CI)[1]	P value
CYP19A1 rs2899470						
GG	90	34.35	120	43.64	1.0 (Ref.)	-
TG	130	49.62	131	47.64	1.32 (0.90-1.94)	0.13
TT	42	16.03	24	8.73	2.33 (1.27-4.33)	0.03
TG + TT	172	65.65	155	56.37	1.48 (1.03-2.13)	0.03
GSTM1						
Present	150	57.25	144	52.36	1.0 (Ref.)	-
Null	112	42.75	131	47.64	0.82 (0.58-1.17)	0.26
GSTT1						
Present	192	73.28	213	77.45	1.0 (Ref.)	-
Null	70	26.72	62	22.55	1.25 (0.83-1.89)	0.26
GSTP1 rs1695						
AA	112	42.75	124	45.09	1.0 (Ref.)	-
AG	118	45.04	128	46.55	1.02 (0.70-1.48)	0.91
GG	32	12.21	23	8.36	1.54 (0.82-2.93)	0.15
AG + GG	150	57.25	151	54.91	1.10 (0.77-1.57)	0.58

[1]Adjusted for body mass index, HOMA-IR, luteinizing hormone, progesterone, estradiol, T-testosterone, fasting plasma glucose, and fasting insulin.

DISCUSSION

Endometriosis is widely accepted as a multifactorial disease; generally, disease pathogenesis can be promoted by a single dominant mutation, resulting in the expression of susceptibility genes. In the present study, we found that *CYP19A1* rs2899470, but not *GSTM1*, *GSTT1*, and *GSTP1* rs1695 polymorphisms, were correlated with increased risk of endometriosis.

CYP19A1 is an important enzyme for estrogen production, and is located on chromosome 15q21.2, which consists of a 30-kb coding area and a 93-kb control area. Polymorphism in the *CYP19A1* gene could alter an individual's susceptibility to endometriosis. Currently, many studies have indicated that *CYP19A1* polymorphisms are associated with endometriosis development, and have revealed that these genetic polymorphisms are correlated with high levels of estrogen (Haiman et al., 2007; Allen et al., 2008; Hosseini et al., 2016). rs2899470 is located in the intron portion of the *CYP19A1* gene, and is associated with levels of steroid hormones, such as testosterone and estrogen (Bulun et al., 2003; Barrett et al., 2005). Previous studies have indicated that *CYP19A1* rs2899470 is associated with circulating estrogen levels, serum E_2/T ratio, and E_2 levels (Jiang et al., 2010).

E_2/T ratio is an important indicator of aromatase activity, which is responsible for aromatization of androgens into estrogens (Jin et al., 2009). *CYP19A1* gene polymorphism is associated with reduced aromatase activity, which can lead to the development of endometriosis (Wang et al., 2010; Zaree et al., 2015; Hosseini et al., 2016; Abu Hashim, 2016). While previous studies have investigated the association between *CYP19A1* and risk of endometriosis, the results were inconsistent (Hur et al., 2007; Vietri et al., 2009; Yang et al., 2010; Szczepańska et al., 2013; Wang et al., 2012, 2014). We have shown that there is a significant correlation between *CYP19A1* rs2899470 and risk of endometriosis in a Chinese population.

Several limitations were present in this study. First, all study subjects were selected from the same hospital, and may not be sufficiently representative of other populations. Second, it is possible that genes apart from *CYP19A1* may also play a role in endometriosis development, and therefore gene-gene interactions should be considered during future analysis.

Further studies with more genes should be included in future studies. Third, the sample size was relatively small in our study, which may have reduced the statistical power of our analysis.

In conclusion, our study suggests that *CYP19A1* rs2899470, but not the *GSTM1*, *GSTT1* and *GSTP1* rs1695, contribute to endometriosis susceptibility in a Chinese population. Further studies with larger sample sizes are needed to verify our results.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

Abu Hashim H (2016). Aromatase Inhibitors for Endometriosis-Associated Infertility; Do We Have Sufficient Evidence? *Int. J. Fertil. Steril.* 10: 270-277.

Abutorabi R, Baradaran A, Sadat Mostafavi F, Zarrin Y, et al. (2015). Evaluation of Tumor Necrosis Factor Alpha Polymorphism Frequencies in Endometriosis. *Int. J. Fertil. Steril.* 9: 329-337.

Allen NE, Key TJ, Dossus L, Rinaldi S, et al. (2008). Endogenous sex hormones and endometrial cancer risk in women in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Endocr. Relat. Cancer* 15: 485-497. http://dx.doi.org/10.1677/ERC-07-0064

Barrett JC, Fry B, Maller J and Daly MJ (2005). Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21: 263-265. http://dx.doi.org/10.1093/bioinformatics/bth457

Bulun SE, Sebastian S, Takayama K, Suzuki T, et al. (2003). The human CYP19 (aromatase P450) gene: update on physiologic roles and genomic organization of promoters. *J. Steroid Biochem. Mol. Biol.* 86: 219-224. http://dx.doi.org/10.1016/S0960-0760(03)00359-5

Haiman CA, Dossus L, Setiawan VW, Stram DO, et al. (2007). Genetic variation at the CYP19A1 locus predicts circulating estrogen levels but not breast cancer risk in postmenopausal women. *Cancer Res.* 67: 1893-1897. http://dx.doi.org/10.1158/0008-5472.CAN-06-4123

Henidi B, Kaabachi S, Mbarik M, Zhioua A, et al. (2015). Glutathione S-transferase M1 and T1 gene polymorphisms and risk of endometriosis in Tunisian population. *Hum. Fertil. (Camb.)* 18: 128-133. http://dx.doi.org/10.3109/14647273.2014.989925

Hosseini E, Mehraein F, Shahhoseini M, Karimian L, et al. (2016). Epigenetic alterations of CYP19A1 gene in Cumulus cells and its relevance to infertility in endometriosis. *J. Assist. Reprod. Genet.* 33: 1105-1113. http://dx.doi.org/10.1007/s10815-016-0727-z

Hur SE, Lee S, Lee JY, Moon HS, et al. (2007). Polymorphisms and haplotypes of the gene encoding the estrogen-metabolizing CYP19 gene in Korean women: no association with advanced-stage endometriosis. *J. Hum. Genet.* 52: 703-711. http://dx.doi.org/10.1007/s10038-007-0174-x

Jin H, Xu CX, Lim HT, Park SJ, et al. (2009). High dietary inorganic phosphate increases lung tumorigenesis and alters Akt signaling. *Am. J. Respir. Crit. Care Med.* 179: 59-68.

Jiang J, Tang NL, Ohlsson C, Eriksson AL, et al. (2010). Association of genetic variations in aromatase gene with serum estrogen and estrogen/testosterone ratio in Chinese elderly men. *Clin. Chim. Acta* 411: 53-58. http://dx.doi.org/10.1016/j.cca.2009.09.039

Kerimoglu OS, Yılmaz SA, Pekin A, Nergiz S, et al. (2015). Follicle-stimulating hormone receptor gene polymorphisms in women with endometriosis. *Arch. Gynecol. Obstet.* 291: 1411-1416. http://dx.doi.org/10.1007/s00404-014-3562-4

Kubiszeski EH, de Medeiros SF, da Silva Seidel JA, Barbosa JS, et al. (2015). Glutathione S-transferase M1 and T1 gene polymorphisms in Brazilian women with endometriosis. *J. Assist. Reprod. Genet.* 32: 1531-1535. http://dx.doi.org/10.1007/s10815-015-0554-7

Lamp M, Saare M, Laisk T, Karro H, et al. (2010). Genetic variations in vascular endothelial growth factor but not in angiotensin I-converting enzyme genes are associated with endometriosis in Estonian women. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 153: 85-89. http://dx.doi.org/10.1016/j.ejogrb.2010.07.021

McLeod BS and Retzloff MG (2010). Epidemiology of endometriosis: an assessment of risk factors. *Clin. Obstet. Gynecol.* 53: 389-396. http://dx.doi.org/10.1097/GRF.0b013e3181db7bde

Parazzini F, Esposito G, Tozzi L, Noli S, et al. (2016). Epidemiology of endometriosis and its comorbidities. *Eur. J. Obstet. Gynecol. Reprod. Biol.* pii: S0301-2115(16)30172-5.

Szczepańska M, Wirstlein P, Skrzypczak J and Jagodziński PP (2013). Polymorphic variants of CYP17 and CYP19A and risk of infertility in endometriosis. *Acta Obstet. Gynecol. Scand.* 92: 1188-1193.

Vietri MT, Cioffi M, Sessa M, Simeone S, et al. (2009). CYP17 and CYP19 gene polymorphisms in women affected with endometriosis. *Fertil. Steril.* 92: 1532-1535. http://dx.doi.org/10.1016/j.fertnstert.2008.07.1786

Wang HS, Wu HM, Cheng BH, Yen CF, et al. (2012). Functional analyses of endometriosis-related polymorphisms in the estrogen synthesis and metabolism-related genes. *PLoS One* 7: e47374. http://dx.doi.org/10.1371/journal.pone.0047374

Wang L, Lu X, Wang D, Qu W, et al. (2014). CYP19 gene variant confers susceptibility to endometriosis-associated infertility in Chinese women. *Exp. Mol. Med.* 46: e103. http://dx.doi.org/10.1038/emm.2014.31

Wang XQ, Yu J, Luo XZ, Shi YL, et al. (2010). The high level of RANTES in the ectopic milieu recruits macrophages and induces their tolerance in progression of endometriosis. *J. Mol. Endocrinol.* 45: 291-299.

Yang H, Liu J, Fan Y, Guo Q, et al. (2016). Associations between various possible promoter polymorphisms of MMPs genes and endometriosis risk: a meta-analysis. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 205: 174-188. http://dx.doi.org/10.1016/j.ejogrb.2016.08.015

Yang X, Chen SQ and Liu M (2010). [Association of the CYP19 gene polymorphism with genetic susceptibility to endometriosis]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 27: 692-696.

Zaree M, Shahnazi V, Fayezi S, Darabi M, et al. (2015). Expression levels of PPARg and CYP-19 in polycystic ovary syndrome primary granulosa cells: influence of w-3 fatty acid. *Int. J. Fertil. Steril.* 9: 197-204.