

# CYP17A1 rs743572 polymorphism might contribute to endometriosis susceptibility: evidences from a case–control study

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## Abstract

This case–control study was aimed to evaluate the influence of cytochrome P450 family 17 subfamily A member 1 (*CYP17A1*) gene rs743572 polymorphism for the susceptibility to endometriosis.

Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) was used to genotype rs743572 polymorphism in 143 endometriosis patients and 148 healthy controls. Hardy–Weinberg equilibrium (HWE) test was utilized to detect the representativeness of the study subjects. Association strength was presented by odds ratios (ORs) with corresponding 95% confidence intervals (CIs).

Genotype distribution of rs743572 polymorphism was conformed to HWE test both in case and control groups, revealing the good representativeness of our study subjects. Significantly positive association was discovered between rs743572 TT genotype and endometriosis susceptibility ( $P = .042$ ,  $OR = 1.952$ , 95%  $CI = 1.020–3.736$ ). Rs743572 T allele was more frequently discovered in cases than that in controls, revealing the enhanced susceptibility to endometriosis ( $P = .041$ ,  $OR = 1.407$ , 95%  $CI = 1.014–1.951$ ). Confounding factors (age and body mass index) were utilized to adjust the results, and then we found that the association strength had no significant changes (TT vs CC,  $P = .039$ ,  $OR = 1.961$ , 95%  $CI = 1.023–3.742$ ; T vs C,  $P = .038$ ,  $OR = 1.413$ , 95%  $CI = 1.016–1.957$ ). But we failed to find any obvious association of rs743572 genotypes with endometriosis stages and characteristics.

T allele of rs743572 polymorphism might act as a risk factor for endometriosis, although it had no effects on the disease stages and basic features.

**Abbreviations:** 5'-UTR = 5'-untranslated region, BMI = body mass index, CIs = confidence intervals, CYP17 = Cytochrome P450 family 17, CYP17A1 = cytochrome P450 family 17 subfamily A member 1, ESHRE = European Society of Human Reproduction and Embryology, HWE = Hardy–Weinberg equilibrium, MRI = magnetic resonance imaging, ORs = odds ratios, PCR–RFLP = polymerase chain reaction–restriction fragment length polymorphism, SNP = single-nucleotide polymorphism.

**Keywords:** CYP17A1, endometriosis, polymorphism

## 1. Introduction

Endometriosis is a common gynecologic disease of females during childbearing period.<sup>[1]</sup> It is induced by the endometrial tissues which normally grows inside the endometrium and also grows outside of it. Its morbidity is increased in recent years.<sup>[2,3]</sup> Although a part of endometriosis patients had no symptoms, pelvic pain and infertility are the main symptoms of endometriosis.<sup>[4]</sup> Endometriosis is not a malignant disease, but it has similar biological behaviors with malignant tumors in invasion,

metastasis, and recurrence.<sup>[5]</sup> It contains 3 stages: adhesion, invasion, and angiogenesis.<sup>[6]</sup> Several hypotheses have been presented to explain the development of endometriosis. The widely accepted theories include retrograde menstruation (also known as transplanted theory), immunodeficiency, coelomic metaplasia, and müllerianosis.<sup>[7–11]</sup> Despite the unclear pathogenesis of endometriosis, it has been considered as a complex process caused by various factors.<sup>[12–16]</sup> Hormone and cytokine activity, immune inflammation reaction, and genetic factors might all participate in the development of endometriosis.

It has been found that endometriosis is an estrogen-dependent disease.<sup>[17,18]</sup> Disorders of estrogen metabolism might be closely associated with the development of endometriosis.<sup>[19,20]</sup> Thus, enzymes which regulate the synthesis and metabolism of estrogen might participate in endometriosis onset. Cytochrome P450 family 17 (CYP17) is one of the key enzymes for steroidogenic pathway which produces estrogen.<sup>[21]</sup> In human, it is encoded by *CYP17A1* (CYP17 subfamily A member 1) gene which is located at chromosome 10q24.32. CYP17 plays its 17 $\alpha$ -hydroxylase and 17,20-lyase activities in endoplasmic reticulum.<sup>[22]</sup> Polymorphisms of *CYP17A1* gene might alter the expression level and activity of CYP17, then resulting in the disorders of estrogen metabolism. Therefore, *CYP17A1* polymorphisms might act as the risk factors for endometriosis. A widely studied single-nucleotide polymorphism (SNP) in the 5'-UTR (untranslated

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**Table 1**  
**Basic characteristics.**

Characteristics	Case n=143 (%)	Control n=148 (%)	P
Age, y	36.42 ± 7.63	36.97 ± 8.37	.806
BMI, kg/m <sup>2</sup>	23.14 ± 4.23	22.97 ± 3.85	.385
Age of menarche, y	13.58 ± 1.42	13.94 ± 1.32	.612
Menstrual cycle, d	28.01 ± 3.22	30.62 ± 5.24	.048
Intrauterine device use	37 (25.87)	24 (16.22)	.043
Oral contraceptive use	37 (25.87)	27 (18.24)	.116
Coffee	63 (44.06)	69 (46.62)	.660
Tea	73 (51.05)	91 (61.49)	.073
Drinking	13 (9.09)	19 (12.84)	.307
Cigarette smoking	20 (13.99)	7 (4.73)	.007
Secondhand smoking	57 (39.86)	35 (23.65)	.003

Drinking, alcohol consumption ≥50 g/time, and drinking frequency >2 times/wk; smoking, cigarette consumption ≥1/d, and lasting >6 months.

region) of *CYP17A1* gene is significantly associated with the gene expression.<sup>[2,3]</sup>

In the present study, we analyzed the effects of *CYP17A1* rs743572 SNP on the susceptibility, stages, and basic characteristics of endometriosis, so as to certify the mechanism of *CYP17A1* gene for endometriosis.

**2. Materials and methods**

**2.1. Study subjects**

The present study was a retrospective case–control study. During January 2012 to July 2016, patients with endometriosis who diagnosed in Affiliated Zhongshan Hospital of Dalian University were enrolled as cases. The patients were diagnosed by 2 pathologists using B-scan ultrasonography, magnetic resonance imaging (MRI), and pathological examinations. Classification of endometriosis was according to the European Society of Human Reproduction and Embryology (ESHRE) guidelines.<sup>[24]</sup> Healthy controls were recruited from the individuals who received healthy checkup in the same hospital from May 2015 to July 2016. The selection of healthy control was a prospective process, and the healthy individuals were matched with the cases both in age distribution and body mass index (BMI). Controls received gynecologic examinations and had no gynecologic disorders. All of the subjects had no systemic diseases, inflammatory disorders, and none of them took hormone drugs or immunosuppressive drugs in recent 3 months. All the subjects belong to Chinese Han population.

Ethic committee of Affiliated Zhongshan Hospital of Dalian University gave the admission of this study. Written informed consent was signed by every participant after understanding the study process. Demographic characteristics were collected through the questionnaire.

**Table 2**  
**Association between rs743572 and endometriosis susceptibility.**

Genotype/allele	Case n=143(%)	Control n=148(%)	P	OR (95%CI)	P*	OR (95% CI)*
CC	37 (25.87)	50 (33.78)	—	—	—	—
TC	67 (46.85)	71 (47.97)	.378	1.275 (0.743–2.189)	.369	1.281 (0.748–2.191)
TT	39 (27.27)	27 (18.24)	.042	1.952 (1.020–3.736)	.039	1.961 (1.023–3.742)
C	141 (49.30)	171 (57.77)	—	—	—	—
T	145 (50.70)	125 (42.23)	.041	1.407 (1.014–1.951)	.038	1.413 (1.016–1.957)
P <sub>HWE</sub>	0.453	0.838				

\* Adjusted by age and body mass index.

**2.2. Genotyping method**

Peripheral venous blood was collected from every subject in case and control group using anticoagulative tubes which contain EDTA. Genomic DNA was extracted by the TIANamp Blood DNA Kit (TIANGEN, Beijing).

*CYP17* rs743572 SNP was amplified using the primers of 5'-CATTGCGACTCTGGA GTC-3' (forward) and 5'-AGGCTCT TGGGGTACTTG-3' (reverse). PCR process included initial denaturation at 94°C for 5 minutes, 40 cycles of denaturation at 94°C for 60 seconds, annealing at 57°C for 50 seconds, extension at 72°C for 60 seconds, then followed the final extension at 70°C for 10 minutes. PCR products of rs743572 were digested by *MspA1I* overnight at 37°C.

**2.3. Statistical analysis**

Calculations were performed by PASW 18.0. Statistic significance was existed when *P* < .05. Hardy–Weinberg equilibrium (HWE) test was utilized to detect the goodness-of-fit of the study subjects. Continuous variables were evaluated by *t* test or nonparametric test. Chi-square test or Fisher exact test was utilized to assess the categorical variables. Genotype and allele frequencies were calculated by direct counting. Association between *CYP17* SNPs and endometriosis susceptibility was assessed by Chi-square test, and adjusted to age and BMI using logistic regression analysis. The results were presented by odds ratios (ORs) with 95% confidence intervals (CIs).

**3. Results**

**3.1. Basic characteristic of subjects**

Endometriosis patients were classified into I (22/143), II (18/143), III (82/143), IV (21/143) stages. Age distribution and BMI had no significant difference between case and control groups (Table 1; *P* > .05). Besides, age of menarche, oral contraceptive use, coffee, tea, and alcohol consumption all had similar trend between endometriosis patients and healthy controls (*P* > .05 for all). Although the menstrual cycle was significantly longer in controls than that in cases (*P* = .048), intrauterine device use, smoking, and secondhand smoking were more frequently discovered in patients than that in controls (*P* < .05).

**3.2. Association between rs743572 and endometriosis susceptibility**

Genotype and allele distributions of rs743572 SNP were conformed to HWE test both in case and control groups (Table 2; *P* > .05), demonstrating that the study subjects could represent the general population.

**Table 3**  
Effects of rs743572 for characteristics of endometriosis patients.

Characteristics	CC n=37(%)	TC n=67(%)	TT n=39(%)
Age, y	35.94±8.17	36.37±7.88	36.95±7.95
BMI, kg/m <sup>2</sup>	22.95±4.86	23.06±4.33	23.35±4.47
Age of menarche, y	13.14±1.46	13.52±1.39	13.74±1.50
Menstrual cycle, d	29.85±4.14	28.78±3.94	27.14±3.78
Intrauterine device use	9 (24.32)	16 (23.88)	12 (30.77)
Oral contraceptive use	9 (24.32)	17 (25.37)	11 (28.21)
Coffee	16 (43.24)	29 (43.28)	18 (46.15)
Tea	19 (51.35)	34 (50.75)	20 (51.26)
Drinking	3 (8.11)	6 (8.96)	4 (10.26)
Smoking	5 (13.51)	9 (13.43)	6 (15.38)
Secondhand smoking	14 (37.84)	27 (40.30)	16 (41.03)

CC, CT, and TT genotype frequencies of rs743572 SNP were 25.87%, 46.85%, 27.27% in cases and 33.78%, 47.97%, 18.24% in controls, respectively. In comparison with CC genotype, TT genotype was significantly associated with increased susceptibility to endometriosis ( $P=.042$ ,  $OR=1.952$ ,  $95\% CI=1.020-3.736$ ). When adjusted by confounding factors, the association strength had no distinct alteration ( $P=.039$ ,  $OR=1.961$ ,  $95\% CI=1.023-3.742$ ). Obviously higher frequency of rs743572 T allele has been discovered in case group than that in control group ( $P=.041$ ), indicating that this allele was significantly associated with enhanced endometriosis susceptibility ( $OR=1.407$ ,  $95\% CI=1.014-1.951$ ). After adjustment, the association was still statistically significant ( $P=.038$ ,  $OR=1.413$ ,  $95\% CI=1.016-1.957$ ).

**3.3. Effects of rs743572 genotype on characteristics of endometriosis patients**

In endometriosis patients, age of menarche was increased in TC and TT genotype carriers, and menstrual cycle was decreased in TC and TT genotype carriers, but it had no significant difference (Table 3;  $P>.05$ ). Besides, no significant distinct has been found in other characteristics among patients carrying rs743572 genotypes ( $P>.05$ ).

**3.4. Stratified analysis based on the stages of endometriosis**

Genotype distributions of rs743572 SNP in different stages of endometriosis were shown in Table 4. Genotype and allele frequencies had no significant difference between 4 endometriosis stages ( $P>.05$ , data not shown). We carried out the stratified analysis based on the endometriosis stages. Nevertheless, we failed to find any significant association between rs743572 and endometriosis susceptibility in every stage ( $P>.05$ , data not

**Table 4**  
Genotype distributions in different stages of endometriosis.

Stages	Genotype			Allele	
	CC	TC	TT	C	T
I n=22	8 (36.36)	8 (36.36)	6 (27.27)	24 (54.55)	20 (45.45)
II n=18	3 (16.67)	10 (55.56)	5 (27.28)	16 (44.44)	20 (55.56)
III n=82	22 (26.83)	37 (45.12)	23 (28.05)	81 (49.39)	83 (50.61)
IV n=21	3 (14.29)	12 (57.14)	6 (28.57)	18 (42.85)	24 (57.14)

shown). These results suggested that rs743572 had no significant effects on the severity and susceptibility of endometriosis.

**4. Discussion**

CYP17 is the rate-limiting enzyme for the biosynthesis of steroid hormones including estrogen. Abnormal expression level of estrogen was associated with the occurrence and development of endometriosis. Mutations in *CYP17A1* gene were correlated with hormone expression, and then contributed to the onset of endometriosis, breast, and prostate cancers.<sup>[2,3]</sup> CYP17 expression level is upregulated in patients with endometriosis than that in controls.<sup>[2,3]</sup> It has been considered that rs743572 SNP is related to the expression of CYP17; T allele might induce the upregulation of CYP17 in patients with prostate cancer.<sup>[2,5]</sup> However, the effects of rs743572 on endometriosis were not clear.

In present study, significantly higher rs743572 TT genotype frequency in case group suggested 1.952 times increased susceptibility to endometriosis. When adjusted by basic characteristics, the association strength was elevated to 1.961. Besides, 1.407 times enhanced endometriosis susceptibility was significantly associated with rs743572 T allele. Confounding factors were used to adjust the result, and then we found that correlation between rs743572 T allele and endometriosis susceptibility was increased to 1.413-fold. The conclusions were in line with the previous studies. Bozdag et al found that homozygote mutation of rs743572 had higher frequency in endometriosis patients.<sup>[2,6]</sup> Szczepańska et al found that rs743572 was positively correlated with endometriosis risk.<sup>[2,7]</sup> The positive association has also been discovered by Hsieh et al in a Chinese population,<sup>[2,8]</sup> although other studies indicated that rs743572 SNP was not associated with the risk of endometriosis.<sup>[2,9,30]</sup> Meanwhile, no evidence has been found in the meta-analysis for the significant association between rs743572 and endometriosis risk.<sup>[3,1]</sup> These difference might be caused by different populations, relatively small sample size or other factors.

In present study we found that menstrual cycle, intrauterine device use, smoking, and secondhand smoking might act as risk factors for endometriosis. Menstrual cycle changes in the present study were similar to thereof in endometriosis or adenomyosis patients.<sup>[3,2]</sup> Adjustment result demonstrated that basic characteristics had tiny effects on endometriosis. These tiny effects combined with genetic polymorphisms contributed to the endometriosis susceptibility. Soon after, we evaluated the distributions of basic characteristics in patients carrying different rs743572 genotypes. TT genotype carriers had the shortest menstrual cycle, highest frequencies of intrauterine device use, smoking and secondhand smoking exposure, but the differences were not significant. We suggested that rs743572 might had no significant effects on the medical histories, such as age of menarche or menstrual cycle. There was no previous study focused on this topic; it should be verified in future.

In addition, we also analyzed the effects of rs743572 SNP on the severity of endometriosis. No significant effect has been discovered in rs743572 on the endometriosis severity. Stratified analysis based on the disease stages also failed to find any significant association between rs743572 and endometriosis susceptibility. Absent of statistical significance for the results probably induced by the small sample size. But a noteworthy correlation has been discovered between rs743572 and endometriosis severity in a Turkey population.<sup>[2,6]</sup>

Several limitations should be admitted in present study. First, polymorphism distribution was diverse in different populations, thus our results might not be applied for other populations. Second, CYP17 expression level and activity should be detected in further studies. Third, endometriosis is a complex disease, but interactions between gene and environment factors were not performed in this study. To certify the endometriosis pathogenesis, next studies with enlarged sample size, population number, and other topic are needed in the future.

In conclusion, rs743572 T allele might predict high susceptibility to endometriosis, but it had no significant influence on characteristics and severity of endometriosis.

### Author contributions

**Conceptualization:** Lili Cong.

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**Formal analysis:** Lili Cong.

**Funding acquisition:** Qiang Fu.

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**Project administration:** Qiang Fu.

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**Validation:** Tianming Gao.

**Visualization:** Tianming Gao.

**Writing – original draft:** Tianming Gao.

**Writing – review and editing:** Tianming Gao.

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