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ARTICLE

Association of an oestrogen receptor gene polymorphism in Chinese Han women with endometriosis and endometriosis-related infertility

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Abstract Endometriosis is a steroid-dependent complex disease. The oestrogen receptor plays an important role by mediating oestrogen action and eutopic or ectopic endometrium development. This study investigated whether single-nucleotide polymorphisms in the genes for oestrogen receptor 1 (*ESR1*) and oestrogen receptor 2 (*ESR2*) are associated with endometriosis and endometriosis-related infertility. The participants included 157 infertile and 155 fertile endometriosis women as well as 92 women with primary infertility and 265 fertile women as controls. The iPLEX Gold system (MassARRAY system, Sequenom) was used for genotyping of *ESR1* and *ESR2*. Statistical analysis showed that *ESR1* (rs3798573 A/G) was significantly associated with endometriosis and endometriosis-related infertility ($P = 0.011$, $P = 0.009$). No association was found with *ESR1* (rs1159327 A/G, rs3020348 A/C) and *ESR2* (rs17179740 A/G) either for endometriosis or endometriosis-related infertility. According to the revised American Fertility Society classification, all of the detected single-nucleotide polymorphisms had no association with endometriosis in stage I–II or in stage III–IV. The results suggest that the *ESR1* polymorphism rs3798573 A/G is associated with increased risk of endometriosis and endometriosis-related infertility in Han women from central China.

KEYWORDS: endometriosis, ESR1 gene, ESR2 gene, infertility, polymorphisms

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Introduction

Endometriosis is an oestrogen-dependent inflammatory benign gynaecological disease that is defined as a condition in which functional endometrial tissue is present outside the uterus. The main clinical manifestations are dysmenorrhoea, chronic pelvic pain, pain during intercourse and infertility (Bulun, 2009). Up to 10% women of reproductive age suffer from this disease, in which 35—50% patients are accompanied by infertility or pelvic pain or both (Cramer et al., 1986; Giudice and Kao, 2004). Although the aetiology of endometriosis is still unclear, various evidence has been found to show that endometriosis is a complex disease, which is affected by inflammation, hormonal regulation and genetic and environmental factors (Falconer et al., 2007; Olive and Schwartz, 1993; Rizner, 2009).

Oestrogen plays a significant role in endometriosis, which enhances the survival or maintenance of endometriotic tissues (Bruner et al., 1997; Ryan and Taylor, 1997). Application of gonadotrophin-releasing hormone agonists can regress endometrial implants and reduce pelvic pain symptoms through inhibiting oestrogen concentrations. The biological effects of oestrogens are mediated by oestrogen receptors, which are members of the nuclear hormone receptor superfamily and react as ligand-activated transcription factors (Ascenzi et al., 2006). Oestrogen receptors include two isoforms: ESRα and ESRβ, each of which is encoded by only one gene, *ESR1* and *ESR2* respectively. Several studies have revealed that the expression of ESRα and ESRβ is different between normal endometrium and ectopic tissues. ESRβ expression suppresses ESRα expression, which results in remarkably high ESRβ/ESRα ratio in endometriotic cells. This aberrant ESRβ/ESRα ratio was associated with progesterone resistance and inflammation, leading to pelvic pain and dysmenorrhoea (Brandenberger et al., 1999; Rizner, 2009).

The relationship between endometriosis and infertility remains unclear (Vercellini et al., 2009). Some investigators have suggested variant mechanisms, including suboptimal embryo quality, impaired ovulation and decreased endometrial receptivity (Kim et al., 2007; Zanatta et al., 2010). Up to now, a number of polymorphic sites in *ESR1* and *ESR2* have been identified to build some associations with endometriosis, endometriosis-related infertility and unexplained infertility in different populations (Altmäe et al., 2007; Guo, 2006; Tempfer et al., 2009). However, the results are controversial.

Therefore, it is meaningful to discuss the relationship between oestrogen receptors and endometriosis or endometriosis-related infertility. A prior pooling-based genome-wide study comparing endometriosis patients and controls (W. Wang, Y. Li, S. Li, Z. Wu, M. Yuan, T. Wang, M. Maitituoheti, D. Ma , S. Wang, unpublished data) identified two endometriosis-related oestrogen receptor single-nucleotide polymorphisms (SNP) in *ESR1* (rs3798573 A/G) and *ESR2* (rs17179740 A/G) using the Affymetrix Genome-Wide Human SNP Array 6.0. Additionally, another two *ESR1* SNP (rs1159327 A/G and rs3020348 A/C) from the results of a Japanese genome-wide association study in endometriosis were also selected for detection in patients from central China (Uno et al., 2010).

Materials and methods

Patients

A total of 669 women who underwent surgery between 2007 and 2011 at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology were selected to participate in this study. All patients were diagnosed by laparoscopy or laparotomy and the study includes 312 women with endometriosis and 357 women without endometriosis as controls. According to the revised American Fertility Society (AFS) classification, 52 (16.67%) had minimal or mild endometriosis (stage I—II) and 260 (83.33%) had moderate or severe endometriosis (stage III—IV). The control patients were operated for hysteromyoma or hydrosalpinx. There were 157 (50.32%) endometriosis women with infertility and 92 (25.77%) controls with primary infertility. All the patients signed informed consent forms. Ethics approval (reference no. S462) for this study was obtained from Tongji Medical College, Huazhong University of Science and Technology on 26 July 2011.

In the 6 months prior to surgery, the participating patients received no hormonal therapy and none had a normal or ectopic pregnancy. None of the patients had anomalies of genital tract disease or malignant disease history.

DNA extraction and genotyping of *ESR1* and *ESR2*

Whole-blood samples were collected from patients in this study using EDTA-K treated tubes. Genomic DNA samples were extracted from leukocytes in blood with QIAamp DNA Blood Mini Kit (Qiagen, Düsseldorf, Germany).

Four SNP were selected for genotyping: rs3798573 A/G, rs1159327 A/G, rs3020348 A/C and rs17179740 A/G. The first three are located separately in intron 6 and the 5′-untranscribed region (UTR) of *ESR1* and the fourth was located in the 5′-UTR of *ESR2*. The IPLEX Gold system was used for genotyping (MassARRAY System; CapitalBio Corporation, Beijing, China). PCR primers were designed by Genotyping Tools and MassARRAY Assay Design software (Sequenom) and synthesized by Invitrogen Biotech (Shanghai, China) (Supplementary Table 1, available online only). After amplification, purification, extension and resin cleaning, the PCR products were tested using 384 format SpectroCHIP microarrays. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was applied for data acquisitions (Sequenom). TYPER 4.0 software was used for data analysis (Sequenom). In order to control quality, 10% of the samples were duplicated for genotyping and concordance rate was 100%. All tested SNP in the whole samples had a call rate $\geq$99% (Syrmis et al., 2011).

Statistical analysis

Statistical analysis of characteristics between endometriosis patients and controls were carried out using two methods: non-parametric Kruskal—Wallis test for age, age of menarche, menstrual cycle and menstrual period and chi-squared test for infertility rate. The Hardy—Weinberg equilibrium was assessed individually for endometriosis

Table 1 Characteristics of patients with endometriosis and controls.

Characteristic	*Controls (n = 357)*	*Endometriosis (n = 312)*	*P-value*
Age (years)	34.23 ± 8.66	33.54 ± 7.52	NS
Age at menarche (years)	13.38 ± 1.60	13.24 ± 1.36	NS
Menstrual cycle length (days)	29.54 ± 4.51	29.52 ± 3.65	NS
Menstrual period length (days)	5.69 ± 1.70	5.98 ± 1.51	0.015
Infertility	92 (25.77)	157 (50.32)	0.0001

Values are mean ± SD or *n* (%). NS = not statistically significant.

samples and controls by chi-squared test. Genotype distribution and allele frequency between endometriosis patients and controls were evaluated by Cochran–Armitage trend test. Multiple tests were corrected by Bonferroni correction. Odds ratios (ORs) and corresponding 95% confidence intervals (95% CI) were estimated by logistic regression analysis. Based on $\alpha = 0.05$ and $1 - \beta = 80\%$, MedCalc version 12.2 for Windows (MedCalc Software, Belgium) was used to calculate the sample size of each group. The minimum target sample size of each group was 292 and the cases and controls in this study were 312 and 357, respectively.

All statistical analyses were done using Statistical Package for Social Sciences version 18.0 for Windows (SPSS, Chicago, IL, USA) and R version 2.14.1 (R Development Core Team). The association study of *ESR1* haplotypes and endometriosis or endometriosis-related infertility was performed using Haploview version 4.1 (www.hapmap.org). $P < 0.05$ was considered statistically significant.

Results

The characteristics of the 312 endometriosis women and 357 control women enrolled in this study are shown in **Table 1**. The mean age of the endometriosis women (33.54 ± 7.52 years) was similar to that of the controls (34.23 ± 8.66 years). There was no statistical difference in age at menarche between endometriosis women (13.24 ± 1.36 years) and controls (13.38 ± 1.60 years). However, the menstrual period of endometriosis women (5.98 ± 1.51 days) was longer than controls (5.69 ± 1.60 days, $P = 0.015$). After adjusting for menstrual period length, no difference in menstrual cycle length was found between endometriosis women (29.52 ± 3.65 days) and the control group (29.54 ± 4.51 days). The percentage of women with infertility in the endometriosis group (50.32%) was higher than in the control group (25.77%, $P < 0.0001$).

The genotypes of all of the SNP and the results of the association analysis in the endometriosis and control groups are shown in **Table 2**. Control women and endometriosis

Table 2 Genotype and allele frequencies of *ESR1* and *ESR2* polymorphisms in endometriosis women and controls.

	n	*Alleles (1/2)*	*Genotypes (n)*			*Alleles (n)*		*OR (95% CI)*	*P-value*
			1/1	*1/2*	*2/2*	*1*	*2*		
ESR1 rs3798573									
Controls	357	A/G	140	154	63	434	280		
Endometriosis	312		142	138	32	422	202	1.35 (1.08–1.69)	0.011
Stage I–II	52		25	24	3	74	30	1.59 (1.02–2.50)	NS
Stage III–IV	260		117	114	29	348	172	1.31 (1.03–1.65)	0.032
ESR1 rs1159327									
Controls	357	A/G	23	116	218	162	552		
Endometriosis	312		12	113	187	137	487	1.04 (0.81–1.35)	NS
Stage I–II	52		1	19	32	21	83	2.03 (1.08–3.81)	NS
Stage III–IV	260		12	94	154	118	402	1.05 (0.80–1.38)	NS
ESR1 rs3020348									
Controls	357	A/C	32	136	189	200	514		
Endometriosis	312		18	125	169	161	463	1.12 (0.88–1.43)	NS
Stage I–II	52		3	20	29	26	78	1.17 (0.73–1.87)	NS
Stage III–IV	260		15	105	140	135	385	1.11 (0.86–1.43)	NS
ESR2 rs17179740									
Controls	357	A/G	10	132	215	152	562		
Endometriosis	312		16	97	199	129	495	1.04 (0.80–1.35)	NS
Stage I–II	52		3	9	40	15	89	1.61 (0.90–2.85)	NS
Stage III–IV	260		13	88	159	114	406	0.96 (0.73–1.27)	NS

Statistical comparisons are with controls. NS = not statistically significant.

Table 3 Genotype and allele frequencies of *ESR1* and *ESR2* polymorphisms in infertile endometriosis women and controls with and without infertility.

	n	*Alleles (1/2)*	*Genotypes* (n)			*Alleles* (n)		*OR (95% CI)*	P-*value*[a]	P-*value*[b]
			1/1	*1/2*	*2/2*	*1*	*2*			
ESR1 rs3798573										
Fertile controls	265	A/G	108	112	45	328	202			
Infertile endometriosis	157		79	65	13	223	91	1.51 (1.12–2.04)	0.009	0.003
Infertile controls	92		32	42	18	106	78	0.84 (0.60–1.18)	NS	
ESR1 rs1159327										
Fertile controls	265	A/G	15	85	165	115	415			
Infertile endometriosis	157		8	55	94	71	243	0.95 (0.68–1.33)	NS	NS
Infertile controls	92		8	31	53	47	137	0.81 (0.55–1.19)	NS	
ESR1 rs3020348										
Fertile controls	265	A/C	22	99	144	143	387			
Infertile endometriosis	157		10	66	81	86	228	0.98 (0.72–1.34)	NS	NS
Infertile controls	92		10	37	45	57	127	0.83 (0.57–1.19)	NS	
ESR2 rs17179740										
Fertile controls	265	A/G	9	93	163	111	419			
Infertile endometriosis	157		4	58	95	66	248	1.00 (0.71–1.40)	NS	NS
Infertile controls	92		1	39	52	41	143	0.92 (0.62–1.39)	NS	

NS = not statistically significant.
[a]Statistical comparisons are with fertile controls.
[b]Statistical comparisons are with infertile controls.

women were in Hardy–Weinberg equilibrium separately (Supplementary Table 2, available online only). The genotype and allele frequencies of rs3798573 A/G in *ESR1* revealed a significant association with endometriosis women (OR 1.35, 95% CI 1.08–1.69, $P = 0.011$). Regarding the other SNP in *ESR1* (rs1159327 A/G and rs3020348 A/C) and *ESR2* (rs17179740 A/G), no statistical difference was found between endometriosis women and controls.

According to the revised AFS classification, endometriosis was divided into two stages to evaluate the relationship between SNP and disease classification (**Table 2**). After application of Bonferroni correction, according to revised AFS classification, no association was found between any of the four SNP and endometriosis.

The relationships between *ESR1* or *ESR2* SNP and endometriosis or endometriosis-related infertility are shown in **Table 3**. A significant association was found between the rs3798573 A/G polymorphism and endometriosis-related infertility ($P = 0.009$). The relative risk was 1.51 for endometriosis-related infertility compared with the fertile controls (95% CI 1.12–2.04). In comparison with infertile controls, the result showed an obvious relationship between endometriosis and *ESR1* rs3798573 A/G ($P = 0.003$). No difference was found between infertile controls and fertile controls. Considering the rs1159327 A/G, rs3020348 A/C and rs17179740 A/G polymorphisms, no association was found between these SNP and endometriosis women with or without infertility (**Table 3**). The haplotype analysis was performed using haploview software version 4.1 and showed that rs1159327 A/G and rs3020348 A/C had strong linkage disequilibrium. However, the haplotype (G/C) showed no association with risk of endometriosis or endometriosis related infertility. The linkage disequilibrium structure is shown in Supplementary Figure 1 (available online only).

Discussion

Analysis of genetic polymorphisms of candidate genes have been regarded as useful for discovering associations between genes and complex disease. Endometriosis is an oestrogen-dependent multigenetic disease and several sex steroid-related genes have been detected to have associations with the disease (Falconer et al., 2007).

Recent studies on *ESR1* and *ESR2* polymorphisms in different populations with endometriosis and endometriosis-related infertility have mainly converged on *ESR1*-$(TA)_n$, ERα-*Pvu*II, ERα-*Xba*I, ERβ-*Alu*I, ERβ-*Rsa*I and *ESR2*-$(CA)_n$, all of which had been repeatedly investigated in different races and sample sizes using various techniques (Georgiou et al., 1999; Hsieh et al., 2007; Lamp et al., 2011). Although the conclusions are discordant, they supply characteristic oestrogen receptor mutation markers for risk of endometriosis under various genetic backgrounds. To improve the study of typical oestrogen receptor polymorphisms and susceptibility to endometriosis in Chinese women, the present study chose for investigation four polymorphisms based on genome-wide scans.

In the analysis of participant characteristics, no differences were detected in age, age at menarche and menstrual cycle between two groups. The endometriosis women showed not only a longer menstrual period but also a notably higher infertile proportion than controls, which conforms to the clinical manifestation and epidemiological observations (Giudice and Kao, 2004).

In *ESR1*, three SNP were detected: rs3798573 A/G in intron 6 and rs1159327 A/G and rs3020348 A/C in the 5′-UTR. Chiefly, rs3798573 A/G was significantly associated with endometriosis, which was in accord with the result of the unpublished previous microarray results. The results also showed a relationship between rs3798573 A/G and endometriosis-related infertility, but this polymorphism had no association with primary infertility. Regarding another two SNP (rs1159327 A/G, rs3020348 A/C), no association with risk of endometriosis or endometriosis-related infertility was found. Inconsistently, in Japanese women, two SNP (rs1159327 A/G and rs3020348 A/C) were shown to be correlated with endometriosis (Uno et al., 2010). However, no relationship was found between these two SNP and susceptibility to endometriosis in Australian and UK women (Painter et al., 2010). These conflicting results might be due to ethnical differences or small sample size.

In *ESR2*, the SNP rs17179740 A/G in the 5′-UTR was investigated and no association between this polymorphism and endometriosis or endometriosis-related infertility was found, which was opposite to the result of the unpublished previous microarray. Owing to the extra experimental error of the pooling-based microarray, an authentic relationship between SNP and disease would inevitably be affected (Pearson et al., 2007).

This study found that *ESR1* rs3798573 A/G correlated with risk of endometriosis and endometriosis-related infertility, which has not been previously reported. Although rs3798573 A/G is located in intron 6 and causes no amino acid change, recent reports have suggested that genetic mutations in introns might be involved in transcription, splicing and translation efficiency regulation (Carstens et al., 1998; Zhao and Hamilton, 2007). Therefore, this polymorphism may influence the mRNA concentration of *ESR1* by varying transcription regulatory elements, which may induce dysfunction of *ESR1* and promote pathogenesis of endometriosis. Therefore, it is reasonable to explore the role of this polymorphism in the diagnosis and the recurrence of endometriosis.

In conclusion, these results reveal that the *ESR1* polymorphism rs3798573 A/G has an obvious association with endometriosis and endometriosis accompanied by infertility in central China. This previously unreported polymorphism may be considered as one of the important susceptibility markers to assist early diagnosis and therapy in women with high risk of endometriosis and endometriosis-related infertility.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.rbmo.2012.09.007.

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