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RESEARCH ARTICLE

The ESR1 gene in unexplained recurrent spontaneous abortion

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Abstract

Recurrent spontaneous abortion (RSA) is a health problem that affects nearly 1% of fertile couples. However, the underlying etiology and mechanism(s) remain elusive. The aim of this study was to investigate estrogen receptor (ESR) 1 gene polymorphisms for risk association of unexplained recurrent spontaneous abortion (URSA) in the Chinese Han population. The entire coding region of the ESR1 gene was sequenced from 129 URSA patients and 183 healthy controls. There was a significant difference between the G allele and GG genotype distributions, of the ESR1 gene (XbaI) polymorphism, between the URSA and the control groups ($\chi^2 = 14.93$, df = 1, $p < 0.001$, OR = 2.01 95% CI: 1.41–2.88 by allele; $\chi^2 = 12.24$, df = 2, $p = 0.002$ by genotype). The PvuII polymorphism, C allele frequency was higher in RSA than in controls (41.9% vs. 34.7%, respectively). Women carrying C-G haplotype were associated with an increased risk of URSA in this population (permutation test p value = 0.016, OR = 1.76 95% CI: 1.19–2.59). Estrogen receptor 1 gene PvuII and XbaI polymorphisms were associated with URSA in a Chinese Han population. However, independent replication of these associations are necessary to assure veracity.

Abbreviations: RSA: recurrent spontaneous abortion; URSA: unexplained recurrent spontaneous abortion; ESR: estrogen receptor; SNPs: single nucleotide polymorphisms

Keywords

Estrogen receptor 1, genetic polymorphisms, recurrent spontaneous abortion

History

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Introduction

Abortion is the spontaneous loss of pregnancy before the fetus has reached viability. Recurrent spontaneous abortion (RSA) is defined as two or more consecutive pregnancy losses before the 22nd week of gestation and affects approximate 1% of fertile couples [Clifford et al. 1997]. The etiologies of RSA are heterogeneous with reported possible causes including genetic, anatomical, cervical weakness, endocrine, immune, infective agents, and inherited thrombophilic defects [Regan et al. 2003]. The specific cause of RSA remains unknown in over 50% of affected women; these are classified as unexplained recurrent abortion (URSA) [Li et al. 2002].

Estrogen, one of two important female sex steroid hormones secreted by the ovary, has been estimated to play a crucial role during the progression of preimplantation and pregnancy maintenance [Albrecht et al. 2000; Schindler 2005]. Estrogen acts by binding to estrogen receptors (ESR) 1 and 2; both receptor subtypes are members of the nuclear receptor protein superfamily of transcription factors [Kuiper et al. 1996]. A study on female ESR1 knockout mice reported anovulation and infertility [Hewitt and Korach 2003].

Estrogen receptor polymorphisms are associated with multiple reproductive traits including endometriosis [Luisi et al. 2006; Xie et al. 2009], premature ovarian failure [Bretherick et al. 2008; Yoon et al. 2010], spermatogenic defect, and uterine leiomyoma [Al-Hendy and Salama 2006; Su et al. 2010]. Researchers have attempted to demonstrate an association between ESR gene polymorphisms and abortion. In 1990, two nearby mutations that led to a silent mutation in codon 87 were associated with spontaneous abortion in women with breast cancer [Lehrer et al. 1990]. However, subsequent studies contradicted these findings [Berkowitz et al. 1994; Taylor et al. 1993]. Recently, a small sample size Brazilian study was conducted and no association was observed with recurrent miscarriage [Alessio et al. 2008]. Nonetheless, a statistically significant association was established between women with at least one spontaneous abortion and single nucleotide polymorphisms (SNPs) rs2234693 (C > T, defined by restriction enzyme PvuII) and rs9340799 (A > G, defined by restriction enzyme XbaI) in intron 1 of ESR1 in the same study in a population of Spanish ancestry. The TA haplotype was implicated to be associated with an increased risk of spontaneous abortion in this population [Pineda et al. 2010]. The aim of this investigation was to determine the relationship of SNPs rs2234693 and rs9340799

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in the ESR1 gene within those individuals presenting URSA in a Chinese Han population.

Results

The allele and genotype distributions of the ESR polymorphisms rs2234693 (C > T, PvuII) and rs9340799 (A > G, XbaI) in URSA subjects and healthy controls are shown in Table 1. The polymorphism rs2234693 C allele frequency was higher in the URSA group than controls (41.9% vs. 34.7%), but this difference was not statistically significant (OR = 1.36 95% CI: 0.98–1.88, $p = 0.069$). No dominant or recessive effect was observed (data not show).

In contrast the allelic and genotypic frequencies for the rs9340799 (A > G, XbaI) polymorphism were significantly different between the URSA group and controls ($\chi^2 = 14.93$, df = 1, $p < 0.001$, OR = 2.01 95% CI: 1.41–2.88 by allele; $\chi^2 = 12.24$, df = 2, $p = 0.002$ by genotype). Both the rs9340799 polymorphism G allele and GG genotype frequencies were greater in the URSA group than controls (35.3% vs. 21.3% by allele, 19.4% vs. 7.7% by genotype). Both the dominant model (AA vs. GG + AG, $p = 0.004$, OR = 1.95 95% CI: 1.23–3.09) and recessive model (GG vs. AG + AA, $p = 0.002$, OR = 2.9 95% CI: 1.44–5.83) showed statistical significance.

Table 2 illustrates the distribution of SNPs rs2234693 and rs9340799 respective haplotypes. Consistent with findings described above, women with the C-G haplotype were associated with an increased risk of URSA in our study population (permutation test p value = 0.016, OR = 1.76 95% CI: 1.19–2.59).

Discussion

The C (rs2234693, PvuII) and G (rs9340799, XbaI) allelic frequency in women with greater than two consecutive spontaneous abortions before the 22nd week of gestation was higher than in the healthy controls. Consequently the C-G haplotype was associated with an increased risk of URSA (OR = 1.76), and the T-A haplotype associated with a lower risk of URSA (OR = 0.62).

In a previous Spanish ancestry population study, the TA haplotype of PvuII/XbaI polymorphisms in 177 women with at least one spontaneous abortion (129 with one abortion, 40 with two abortions, and 8 with three or more abortions) was higher than a control group of 442 women with at least one birth. This risk was increased when analysis was restricted to women with two or more spontaneous abortions compared with controls [Pineda et al. 2010].

Several factors could explain the discrepancy of allele and genotype distribution in the ESR1 gene. Differences in subject selection criteria could contribute to conflicting results. Only women with two or more RSA were recruited and assigned to the study's case group, whereas among the total of 177 cases in the previous study [Pineda et al. 2010],

Table 1. Distribution of the ESR1 gene polymorphisms in URSA cases and controls.

	Polymorphism	Cases (%)	Controls (%)	x^2	p Value[a]	OR(95% CI)
rs2234693						
Allele	C	108 (41.9)	127 (34.7)	3.305	0.069	1.36 (0.98–1.88)
	T	150 (58.1)	239 (65.3)			
Genotype	CC (%)	21 (16.3)	20 (10.9)			
	CT (%)	66 (51.2)	87 (47.5)	3.461	0.177	
	TT (%)	42 (32.6)	76 (41.5)			
	Total	129	183			
rs9340799						
Allele	G (%)	91 (35.3)	78 (21.3)	14.933	0.00011	2.01 (1.41–2.88)
	A (%)	167 (64.7)	288 (78.7)			
Genotype	GG (%)	25 (19.4)	14 (7.7)			
	AG (%)	41 (31.8)	50 (27.3)	12.244	0.002	
	AA (%)	63 (48.8)	119 (65.0)			
	Total	129	183			
	AG + GG (%)	66 (51.2)	64 (35.0)	8.160	0.004	1.95 (1.23–3.09)
	AA (%)	63 (48.8)	119 (65.0)			
	AG + AA (%)	104 (80.6)	169 (92.3)	9.518	0.002	2.90 (1.44–5.83)
	GG (%)	25 (19.4)	14 (7.7)			

[a]Evaluated by x^2 test in comparison with the control group. ESR1: estrogen receptor 1; URSA: unexplained recurrent spontaneous abortion.

Table 2. Haplotype frequency of the ESR1 gene polymorphisms in URSA cases and controls.

Haplotype	Case ($n = 129$)	Control ($n = 183$)	x^2	p Value[a]	p Value[b]	OR(95% CI)
T-A	0.491	0.609	8.568	0.003	0.016	0.62 (0.45–0.86)[c]
C-G	0.262	0.169	7.973	0.004	0.022	1.76 (1.19–2.59)[d]
C-A	0.156	0.178	0.497	0.480	0.878	
T-G	0.091	0.044	5.522	0.018	0.078	

[a]Evaluated by x^2 test in comparison with the control group; [b]Evaluated by 10^4 permutation test; [c]T-A vs. C-G + C-A + T-G; [d]C-G vs. T-A + C-A + T-G. ESR1: estrogen receptor 1; URSA: unexplained recurrent spontaneous abortion.

129 (72.9%) had one abortion and 48 (27.1%) had two or more abortions. The risk of spontaneous abortion increased when analysis was limited to the women with two or more abortions compared with controls [Pineda et al. 2010]. The authors highlight that the result should be interpreted with caution as the findings are based on a small sample size. A large sample size is usually required to achieve statistical power in genetic association studies. No association was demonstrated with the URSA group containing 75 cases from a mixed population of Caucasian and African Brazilian [Alessio et al. 2008]. Our study (129 patients) provides a relatively higher power to test the locus' potential association with URSA. In addition, ethnic variation might influence the genetic regulatory pattern. Conflicting reports of the distribution of ESR1 polymorphisms rs2234693 and rs9340799 among different populations have been reported in investigations of other reproductive diseases. The susceptibility to premature ovarian failure was reported to increase with the presence of both PvuII/XbaI haplotype CG (10) and TA [Yoon et al. 2010], and the risk to endometriosis was increased by the presence of both ESR1 polymorphisms PvuII T allele [Georgiou et al. 1999] and PvuII C allele [Hsieh et al. 2007].

The PvuII C allele has been reported to contain a functional binding site for the transcription factor B-myb which augments transcription of a downstream receptor construct 10-fold [Herrington et al. 2002]. The result implies that the presence of the C allele may up-regulate the ESR1 gene. Abnormal expression (both up-regulation and down-regulation) of the ESR1 gene might be associated with pathophysiologic aberrancies involved in URSA. Further functional studies are necessary to understand the etiology of URSA.

In conclusion, we demonstrated a higher frequency of the PvuII C allele and the XbaI G allele in the ESR1 gene in cases when compared with healthy controls in the Chinese Han population. The GG genotype of XbaI polymorphism and the C-G haplotype of PvuII/XbaI polymorphisms are associated with an increased risk of URSA in our study group. Further studies to replicate these associations are necessary. These should include extending the sample size and be applied to different ethnic populations.

Materials and Methods

Subjects

A total of 129 patients with two or more consecutive spontaneous abortions before the 22nd week of gestation and 183 healthy controls were recruited from the Peking Union Medical College, China. Routine clinical assessments were performed and subjects with any known causes of spontaneous abortion, including chromosomal aberrations of partners and the embryos, anatomical factor, immune factor, infective agents, and inherited thrombophilic defects were excluded from analysis. Controls were individuals of proven fertility, with normal menstrual cycles and ovary morphology, without the history of subfertility treatment. The study was approved by the Ethics Committee of Research Institute for Family Planning and informed consent was obtained from all participants.

DNA analysis

Blood samples from URSA patients and healthy controls were collected and stored at -20°C. Genomic DNA was extracted from peripheral blood leukocytes using TIANamp blood DNA kit (Tiangen, Beijing, China). Genetic variants of ESR polymorphisms rs2234693 (C > T, PvuII) and rs9340799 (A > G, XbaI) were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis. The region containing the restriction enzyme sites were amplified by single pairs of genetic specific primers: 5′ GGCTCAAACTACAGGGCTTAAAC 3′ and 5′ TTCATT ACCTCTTGCCGTCT 3′. The PCR products were digested for 16 h at 37°C with 1.5 units of PvuII or XbaI, as recommended by TAKARA (Takara, Otsu, Japan).The 539 bp PCR products were cleaved into 265 bp and 274 bp fragments by PvuII and into 229 bp and 310 bp fragments by XbaI. The enzyme digestion products were separated by electrophoresis on a 3% agarose gel and photographed under ultraviolet illumination.

Genetic analysis

The allele frequency and genotype distribution of polymorphisms rs2234693 and rs9340799 were estimated using the allele-counting method. The URSA group and controls were compared by Pearson χ^2 tests using the Statistical Package for Social Science Version 10.0 (SPSS 10.0). Haplotype association tests were performed using HaploView4.2 software and then adjusted by permutation tests. Statistical significance was recorded when p value < 0.05.

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Declaration of interest

This statement is to certify that all authors have seen and approved the manuscript being submitted, and the article is the authors' original work. There is no conflict of interests. The article has not received prior publication and is not under consideration for publication elsewhere. On behalf of all co-authors, the corresponding author shall bear full responsibility for the submission. This work was supported by the National Basic Research Program of China.

Author contributions

Performed this study, the statistical analysis, and drafting the article: HP, PS; Collected all samples and performed the clinical tests: CL, JW, SZ; Revised this article critically for important intellectual content and final approval of the version to be published: BW, XM.

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