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Int Urogynecol J (2008) 19:1159–1163
DOI 10.1007/s00192-008-0603-1

ORIGINAL ARTICLE

Estrogen receptor alpha polymorphism is associated with pelvic organ prolapse risk

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Received: 12 November 2007 / Accepted: 29 February 2008 / Published online: 3 April 2008
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Abstract Estrogen and estrogen receptors are known to play important roles in the pathophysiology of pelvic organ prolapse (POP). We investigated whether estrogen receptor α (ERα) gene polymorphisms were associated with POP risk by conducting a case-control association study in 88 women with POP and 153 women without POP. Genotypes of the ERα (ESR1) gene polymorphisms (rs17847075, rs2207647, rs2234693, rs3798577, and rs2228480) were determined by polymerase chain reaction, followed by restriction fragment length polymorphism analysis. There was significant difference between women with and those without POP in the distribution of the ESR1 rs2228480 genotypes evaluated. By using multivariable logistic regression, age and ESR1 rs2228480 genotype GA were significantly associated with POP risk. Although the sample size of women with POP studied is small, the present study shows that ERα genotype may be associated with POP risk.

Keywords Pelvic organ prolapse · Estrogen receptor α gene polymorphism · Genotype

Abbreviations

POP	pelvic organ prolapse
ER	estrogen receptor
ERα	estrogen receptor α
ESR1	estrogen receptor α
SNPs	single nucleotide polymorphisms
ICS	International Continence Society
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism

Introduction

Pelvic organ prolapse (POP) is a common gynecological problem [1]. As life expectancy increases, significantly greater number of women will present with POP requiring surgical intervention. Currently, the lifetime risk of undergoing prolapse or continence surgery in the USA is 1 in 11, and up to 30% of patients will require repeat prolapse surgery [2]. Although the pathophysiology of POP is still not completely understood, genetic factors and environmental factors are thought to be involved [1].

Estrogen deficiency, collagen abnormality, vaginal parity, and aging are recognized to be risk factors associated with POP [3–5], but no study has been able to differentiate

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between estrogen effects and aging effects with regard to the risk for POP. Estrogen exerts its effects by interacting with specific estrogen receptors (ERs), the ERα (ESR1) and ERβ (ESR2) [6]. The former isoform is a ligand-activated transcription factor composed of several important domains for hormone binding, DNA binding, and activation of transcription [7]. ERα is an important mediator in signal transduction pathway [7]. The ERα gene is greater than 140 kb, contains eight exons, and is located on chromosome 6q25 [8].

The ability to accurately identify individuals at increased risk for developing POP from a genetic screen would be revolutionary and enormously useful in order to avoid a critical inciting events such as vaginal birth [9]. Single nucleotide polymorphisms (SNPs) are used as a tool for mapping genes responsible for the disease [10] and might be a suitable method for studying disease genes responsible for POP.

The concentrations of estrogen and ER of women who had POP before menopause were lower than those of the control group [11]. The ERα expression was higher in both postmenopausal and premenopausal POP patients than the control groups [12]. Our hypothesis is that genetic variations in the ERα gene might alter the expression of the gene and put women at the risk of POP. In the present study, we investigated a total of five polymorphisms in the ERα gene: ESR1 rs17847075 (exon 1 C/T), ESR1 rs2207647 (exon 1 G/A), ESR1 rs2234693 (intron 1 T/C), ESR1 rs3798577 (exon 8 C/T), and ESR1 rs2228480 (exon 8 G/A) [13–17]. We evaluated the correlations between these polymorphisms and POP risk.

Materials and methods

Study participants

A total of 88 women with POP and 153 women without POP were recruited. Women who were amenorrheic for 1 year or more were considered menopausal. None of the menopausal women in this study had received hormone replacement therapy. The premenopausal women were submitted to blood drawn between days 2 and 5 of the menstrual cycle (proliferative phase) [11]. All subjects in a 45° semiupright position with an empty bladder in a birthing chair were examined during maximum Valsalva maneuver for staging of POP according to the criteria of the International Continence Society [18, 19]. All examinations were performed by a senior gynecologist. The study group (POP group) had POP stage 2 or higher and control group (non-POP group) had POP stage 1 or without any signs of POP. This study was approved by our institutional review board, and written informed consent was obtained from all of the women who participated in this study.

Polymerase chain reaction and typing for ERα gene polymorphisms

The genomic DNA was prepared from peripheral blood using a DNA Extractor WB kit (Wako, Japan). Polymerase chain reactions (PCRs) were carried out in a total volume of 50 µl, containing genomic DNA, 2–6 pmol of each primer, 1X Taq polymerase buffer (1.5 mM $MgCl_2$), and 0.25 units of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA, USA). The primer for the ESR1 rs17847075 (exon 1 C/T), ESR1 rs2207647 (exon 1 G/A), ESR1 rs2234693 (intron 1 T/C), ESR1 rs3798577 (exon 8 C/T), and ESR1 rs2228480 (exon 8 G/A) gene polymorphisms are listed in Table 1 [13–17]. PCR amplification was performed in a programmable thermal cycle GeneAmp PCR System 2400 (Applied Biosystems, Foster City, CA, USA). The cycling conditions are given in Table 1.

The ESR1 rs17847075 (exon 1 C/T) polymorphism was analyzed by PCR amplification, followed by restriction fragment length polymorphism (RFLP) analysis using Msp I (New England Biolabs, Beverly, MA, USA) digestion. The “C” allele of the ESR1 rs17847075 showed up as 198,

Table 1 Sequences of primers used for estrogen receptor alpha genotyping

Polymorphism site	Primer sets	Annealing temperature (°C)	Digestion	Allele size (bp)
ESR1 rs17847075 (exon 1 C/T)	5′-ATGCGCTGCGTCGCCTCTAA-3′ 5′-CTGCAGGAAAGGCGACAGCT-3′	62	Msp I, 37°C	C 198+121+90+53+18 T 288+121+53+18
ESR1 rs2207647 (exon 1 G/A)	5′-GCTTGCTGCTGTCCAGGTACA-3′ 5′-ATGACCATGACCCTCCACACC-3′	62	Msp I, 37°C	A 145 G 114+31
ESR1 rs2234693 (intron 1 T/C)	5′-AGAGGAGTTTGAATATTGAAG-3′ 5′-TAGCTGGTTTCTAATAGACTTA-3′	55	Pvu II, 37°C	C 297 T 165+132
ESR1 rs3798577 (exon 8 C/T)	5′-GGTGAACTGTTCACTGTGGT-3′ 5′-TCTGCCCTACTTTCCCTCTT-3′	55	AlwN I, 37°C	C 106+50 T 156
ESR1 rs2228480 (exon 8 G/A)	5′-GCTCTACTTCATCGCATTCC-3 5′-CCACTAAGAACTGAGCAAGC-3′	58	Btg I, 37°C	A 238 G 174+64

121, 90, 53, and 18 bp on agarose electrophoresis. The "T" allele of the ESR1 rs17847075 presented fragments of 288, 121, 53, and 18 bp. The ESR1 rs2207647 (exon 1 G/A) gene polymorphism was determined by PCR amplification, followed by RFLP analysis using Msp I (New England Biolabs, Beverly, MA, USA) digestion. The "G" allele of the ESR1 rs2207647 was 114 and 31 bp, and the "A" allele of the ESR1 rs2207647 was 145 bp on agarose electrophoresis. The ESR1 rs2234693 (intron 1 T/C) gene polymorphism was analyzed by PCR amplification, followed by RFLP analysis using Pvu II (New England Biolabs, Beverly, MA, USA) digestion. The "C" allele of the ESR1 rs2234693 showed up as 297 bp, and the "T" allele of the ESR1 rs2234693 was 165 and 132 bp on agarose electrophoresis. The ESR1 rs3798577 (exon 8 C/T) gene polymorphism was analyzed by PCR amplification, followed by RFLP analysis using AlwN I (New England Biolabs, Beverly, MA, USA) digestion. The "C" allele of the ESR1 rs3798577 showed up as 106 and 50 bp, and the "T" allele of the ESR1 rs3798577 was 156 bp on agarose electrophoresis. The ESR1 rs2228480 (exon 8 G/A) gene polymorphism was analyzed by PCR amplification, followed by RFLP analysis using Btg I (New England Biolabs, Beverly, MA, USA) digestion. The "A" allele of the ESR1 rs2228480 showed up as 238 bp on agarose electrophoresis. The "G" allele of the ESR1 rs2228480 was 174 and 64 bp.

Statistical analysis

Mann–Whitney test (Wilcoxon rank–sum test) and chi-square test were used for nonparametric ordinal and nonparametric categorical variables, respectively. When the assumption of the chi-square test was violated (i.e., when more than one cell had an expected count of less than 1, or more than 20% of the cells had an expected count of less than 5), Fisher's exact test was used. Genotype frequencies were tested for Hardy–Weinberg equilibrium as a check against genotyping assay problem. A logistic regression model and odds ratios (OR; with 95% confidence intervals (CI)) were used to assess the independent prognostic value of the variables associated with POP risk. All statistical tests were two-sided. P value less than 0.05 was considered statistically significant. All calculations were performed by the Statistical Package for Social Sciences (SPSS for Windows, release 8.0, SPSS Inc., Chicago, IL, USA).

Results

The prevalence of each genotype of the ESR1 rs17847075 (exon 1 C/T) polymorphism was 14.5% CC, 45.7% CT, and 39.8% TT; that of the ESR1 rs2207647 (exon 1 G/A) polymorphism was 13.7% GG, 46.1% GA, and 40.2% AA. The prevalence of each genotype of the ESR1 rs2234693 (intron 1 T/C) polymorphism was 39% TT, 46.1% TC, and 14.9% CC; that of the ESR1 rs3798577 (exon 8 C/T) polymorphism was 19.1% CC, 44.8% CT, and 36.1% TT. Finally, the prevalence of each genotype of the ESR1 rs2228480 (exon 8 G/A) polymorphism was 61% GG, 35.3% GA, and 3.7% AA. The five genotype distributions were in Hardy–Weinberg equilibrium.

We analyzed the influence of five factors on POP: age, parity, body mass index (BMI), menopausal status, and ERα gene polymorphisms (ESR1 rs17847075 (exon 1 C/T), ESR1 rs2207647 (exon 1 G/A), ESR1 rs2234693 (intron 1 T/C), ESR1 rs3798577 (exon 8 C/T), and ESR1 rs2228480 (exon 8 G/A); Table 2). Of these, older age, increased parity, greater BMI, menopausal status, and incidence of ESR1 rs2228480 genotype GA were found to be significantly associated with POP risk.

Univariable logistic regression revealed that older age, increased parity, greater BMI, menopausal status, and incidence of ESR1 rs2228480 genotype GA were significantly associated with POP risk. The ESR1 rs2228480 genotype AA appeared to be a "protective" genotype for POP (OR 0.38, 95% CI 0.05–3.19, P=0.373). The ESR1 rs2228480 genotype GA appeared to be a significant "at-risk" genotype for POP (OR 1.87, 95% CI 1.04–3.38, P=0.038). In view of univariable analyses, we next performed a multivariable logistic regression analysis including all significant or border-line significance risk factors which were found in the univariable analyses. In the model, the final result showed that older age and incidence of ESR1 rs2228480 genotype GA were significantly associated with POP risk (Table 3). That is, older age and incidence of ESR1 rs2228480 genotype GA were significant "at-risk" factors for POP (age, OR 1.05, 95% CI 1.01–1.10, P=0.002; ESR1 rs2228480 genotype GA, OR 2.05, 95% CI 1.05–4.02, P=0.036). Others thought to be potential risk factors, such as parity, BMI, and menopausal status found in the univariable analyses, were not shown to be significant in the multivariable analysis. Their effects seem to be explained by genetic factor and age.

Discussion

To test our hypothesis that sequence variations in the ERα gene are associated with POP risk, we analyzed the exon 1, intron 1, and exon 8 region of the gene in 88 women with POP and 153 women without POP. To our knowledge, this is the first study to evaluate the association between sequence variations in the ERα gene and POP risk. The genotype frequency estimation analysis revealed that the ESR1 rs2228480 genotype GA was more prevalent in

Table 2 Analysis of clinical features and estrogen receptor alpha gene polymorphisms of 241 women with and without pelvic organ prolapse

Variables	Non-POP (*n*=153) No. (%)	POP (*n*=88) No. (%)	*P* value
Age (years)	50.3±9.7	61.1±12.3	<0.001[a]
Parity (median)	3 (0–7)	4 (1–8)	<0.001[a]
Body mass index (kg/m^2)	23.3±3.1	24.2±3.2	0.018[a]
Menopause	69 (45.1)	72 (81.8)	< 0.001[b]
ESR1 rs17847075 (exon 1 C/T)			
CC	23 (15)	12 (13.6)	0.864[b]
CT	71 (46.4)	39 (44.3)	
TT	59 (38.6)	37 (42)	
ESR1 rs2207647 (exon1 G/A)			
GG	21 (13.7)	12 (13.6)	0.982[b]
GA	71 (46.4)	40 (45.5)	
AA	61 (39.9)	36 (40.9)	
ESR1 rs2234693 (intron 1 T/C)			
TT	58 (37.9)	36 (40.9)	0.828[b]
TC	73 (47.7)	38 (43.2)	
CC	22 (14.4)	14 (15.9)	
ESR1 rs3798577 (exon 8 C/T)			
CC	27 (17.6)	19 (31.6)	0.761[b]
CT	70 (45.8)	38 (43.2)	
TT	56 (36.6)	31 (35.2)	
ESR1 rs2228480 (exon 8 G/A)			
GG	102 (66.6)	45 (51.1)	0.015[b]
GA	44 (28.8)	41 (46.6)	
AA	7 (4.6)	2 (2.3)	

Values are mean±standard deviation.
[a] Mann–Whitney test
[b] Chi-square test

women with POP (46.6%) than in women without POP (28.8%). The *P* value of the chi-square test was about 1.5% with a power greater than 74%. The ESR1 rs2228480 genotype GA, therefore, seems to be a risk factor for developing POP.

Many clinicians are confronted by increased numbers of patients requesting elective cesarean sections to preserve the pelvic floor and perineum [20]; therefore, the ability to accurately identify women at risk for POP is needed. Multiparity, obstetric trauma, chronic increased intra-abdominal pressure, aging, estrogen deficiency, and genetic factors are well-known risk factors for developing POP [1, 21–22]. In this study, we assessed the relationship among clinical features, genetic factors, and POP. Our results show that age and ESR1 rs2228480 genotype GA are significant “at-risk” factors for developing POP. This result suggests that genetically susceptible individuals will experience POP.

Table 3 Logistic regression of factors associated with pelvic organ prolapse risk

Covariate	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Age (years)	1.09 (1.06–1.12)	<0.001	1.05 (1.01–1.10)	0.002
Parity	1.74 (1.40–2.16)	<0.001	1.25 (0.96–1.62)	0.100
Body mass index (kg/m^2)	1.09 (1.01–1.93)	0.033	1.08 (0.97–1.20)	0.149
Menopause	5.48 (3.92–10.27)	<0.001	1.68 (0.68–4.13)	0.261
ESR1 rs2228480 (exon 8 G/A)				
GG	1.0		1.0	
GA	1.87 (1.04–3.38)	0.038	2.05 (1.05–4.02)	0.036
AA	0.38 (0.05–3.19)	0.373	0.52 (0.05–5.03)	0.571

CI Confidence interval

Premenopausal women had lower collagen concentration in the pelvic floor in comparison to controls. This difference is not registered after menopause indicating a possible disturbed extracellular matrix in the premenopausal POP [23]. The present study shows that ERα genotype may be associated with POP. The statistical analyses of the clinical features of the recruited patients in case of menopausal status are not clear. In the non-POP group, only half of the patients are menopausal; in the POP group, there are 80%. The genotype frequency estimation analysis revealed that the ESR1 rs2228480 genotype GA was more prevalent in postmenopausal women with POP (47.2%) than in postmenopausal women without POP (29%; $P=0.047$). Our sample size was relatively small, and our data were subjected to a number of uncorrected tests. Therefore, our positive results may represent false-positive findings. A large cohort size is needed to confirm this association. It seems better to avoid such kind of "mix," instead using matched cases for investigation. Further characterizing studies of ERα genotype and their relationship with POP need to be conducted. Besides transcriptional regulation, posttranslational modification of the ERα gene may play a role in the bioactivity of the ERα.

In conclusion, our results show that ERα genotype may be associated with POP risk. Genetic variations in the estrogen receptor alpha gene might play a role in susceptibility in POP. Further study of other SNPs using additional genetic markers might provide new information about the genetic background of POP pathogenesis.

Acknowledgements The authors would like to thank the National Science Council (NSC) of the Republic of China, the China Medical University, and the China Medical University Hospital for financially supporting this research under Contract NSC 96-2815-C-039-035-B, CMU96-035, and DMR-97-076. They also like to thank professor Kuang Fu Cheng, at Biostatistics center of the China Medical University, for his comments on statistical analyses.

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