Original Article

Association between Estrogen Receptor α (ESR1) Gene Polymorphisms and Severe Preeclampsia

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Associations have been reported between estrogen receptor α (ESR1) gene polymorphisms and various pathological conditions, including cardiovascular diseases. Our aim was to investigate whether two polymorphisms of the ESR1 gene (ESR1 c.454 −397T>C: PvuII restriction site and c.454 −351A>G: XbaI restriction site) are associated with preeclampsia. In a case-control study, we analyzed blood samples from 119 severely preeclamptic patients and 103 normotensive, healthy pregnant women using the polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) method. All of the women were Caucasian. There was no association between severe preeclampsia and the PvuII and XbaI ESR1 gene polymorphisms separately. However, with the simultaneous carriage of both polymorphisms, the TT/AA genotype combination was significantly more frequent in severely preeclamptic patients than in healthy control subjects (24.4% *vs.* 9.7%, $p=0.003$), whereas the TT/AG combination was significantly less frequent in the severely preeclamptic group than in the control group (5.0% *vs.* 18.4%, $p=0.002$). According to the haplotype estimation, the homozygous T-A haplotype carriers had an increased risk of severe preeclampsia independent of maternal age, prepregnancy BMI, primiparity and smoking status (adjusted odds ratio [OR]: 4.36, 95% confidence interval [CI]: 1.65–11.53). The GG genotype of the XbaI polymorphism was associated with a lower risk of fetal growth restriction in patients with severe preeclampsia (OR: 0.23, 95% CI: 0.07–0.73). In conclusion, the homozygous T-A haplotype carriers of ESR1 PvuII and XbaI polymorphisms showed an increased risk of severe preeclampsia. In addition, the GG genotype of the XbaI polymorphism decreased the risk of fetal growth restriction in severely preeclamptic patients. (*Hypertens Res* 2007; 30: 205–211)

***Key Words*: estrogen receptor, gene, polymorphism, preeclampsia, cardiovascular disease**

Introduction

Estrogen receptor α (ESR1), a member of the nuclear hormone receptor superfamily, functions as a ligand-activated transcription factor (*1*). ESR1 can be activated not only by estrogen binding but also by growth factors in the absence of estrogen (*2*). It has a molecular weight of approximately 66 kD and contains 595 amino acids (*1*). However, a 46 kD isoform, which is a splice variant, has been reported to be expressed in the plasma membrane of endothelial cells (*3*). ESR1 is expressed in a wide range of tissues, including the

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Table 1. Clinical Characteristics of Severely Preeclamptic Patients and Normotensive, Healthy Pregnant Women

Indicator	Severely preeclamptic patients ($n=119$)	Controls ($n=103$)	Statistical significance (p)
Age (years)*	28 (24–32)	28 (25–31)	>0.05 (n.s.)
Primiparas (n (%))	88 (73.9)	49 (47.6)	<0.001
Gestational age at delivery (weeks)*	33 (30–35)	40 (39–41)	<0.0001
Prepregnancy BMI (kg/m^2)	23.8±4.2	22.6±4.1	<0.05
Smokers (n (%))	12 (10.1)	10 (9.7)	>0.05 (n.s.)
Fetal birth weight (g)	1,565±586	3,493±534	<0.0001
Fetal growth restriction (cases (%))	58 (48.7)	0 (0.0)	<0.0001
Blood pressure, systolic (mmHg)	180±16	123±8	<0.001
Blood pressure, diastolic (mmHg)	112±10	83±6	<0.001

Data are presented as mean±SD for continuous parametric variables, as *median (25–75 percentile) for continuous nonparametric variables, and as number (percent) for categorical variables. BMI, body mass index; n.s., not significant.

vascular endothelial and smooth muscle cells, as well as the human reproductive tissues (*4*, *5*).

The gene encoding ESR1 is located on the long arm of chromosome 6 (6q25.1), and contains 8 exons. Interestingly, the position of its introns is highly conserved among species, suggesting their functional importance (*6*). Several polymorphisms of the ESR1 gene—both single nucleotide polymorphisms and tandem repeats—have been investigated in genetic association studies, and associations were found with various pathological conditions, including cardiovascular disorders such as hypertension, coronary artery disease and stroke (*7–10*). However, the association between blood pressure and variations of the ESR1 gene is controversial (*11*, *12*).

Preeclampsia seems to be a multifactorial disorder with both maternal and paternal genetic components (*13–15*). Increasing evidence suggests that a family history of cardiovascular disorders (chronic hypertension, myocardial infarction, stroke) increases the risk of preeclampsia (*16*, *17*). In addition, a pregnant woman with preeclampsia has an increased risk of developing cardiovascular diseases later in life (*18*).

Given the association between ESR1 gene polymorphisms and cardiovascular disorders, and given that between preeclampsia and cardiovascular diseases, our aim was to investigate the association between ESR1 gene polymorphisms and preeclampsia. We investigated two polymorphisms in the first intron of the estrogen receptor α gene (ESR1 c.454 −397T>C: PvuII restriction site [rs2234693] and c.454 −351A>G: XbaI restriction site [rs9340799]), which are the most frequently studied polymorphisms of the ESR1 gene.

Methods

Study Patients

Enrolled in this case-control study were 119 severely preeclamptic patients and 103 normotensive (blood pressure <140 mmHg systolic and <90 mmHg diastolic), healthy pregnant women with uncomplicated pregnancies. The patients were recruited from the First Department of Obstetrics and Gynecology at the Semmelweis University, Budapest, Hungary. All of the women were Caucasian and resided in the same geographic area in Hungary. Exclusion criteria were multifetal gestation, chronic hypertension, diabetes mellitus, autoimmune disease and renal disease.

Severe preeclampsia was defined by increased blood pressure (≥160 mmHg systolic or ≥110 mmHg diastolic on ≥2 occasions at least 6 h apart) that occurred after 20 weeks of gestation in a woman with previously normal blood pressure, accompanied by proteinuria (≥5 g/24 h) (*19*, *20*). Blood pressure returned to normal by 12 weeks postpartum in each preeclamptic study patient. Fetal growth restriction was diagnosed if the fetal birth weight was below the 10th percentile for the gestational age (*21*).

The study protocol was approved by the Regional, Institutional Committee of Medical Ethics at the Semmelweis University, and each patient provided written informed consent.

Biological Samples and Genotyping

Peripheral blood samples taken for routine laboratory investigations were used for genotyping. Genomic DNA was extracted from EDTA blood samples by a standard phenol-chloroform extraction procedure. The prevalence of ESR1 intron 1 −397T>C (PvuII or IVS1 −397, previously known as IVS1 −401) and −351A>G (XbaI or IVS1 −351, previously known as IVS1 −354) genotypes was investigated. A fragment of 255 base pairs (bp) was generated by a forward primer (5′-CAGGGTTATGTGGCAATGAC-3′) and a reverse primer (5′-TACCTATAAAAATGACAAAATGA AAT-3′) in 10 μl reaction mixture containing polymerase chain reaction (PCR) buffer, 2 mmol/l $MgCl_2$, 0.2 mmol/l dNTP's mix, 0.4 μmol/l of both primers, 1.5 U recombinant Taq DNA Polymerase (Invitrogen, Budapest, Hungary) and 100 ng genomic DNA. The PCR conditions were as follows: initial denaturation at 94°C for 4 min followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and

Table 2. Genotype and Allele Frequencies of the PvuII and XbaI ESR1 Gene Polymorphisms in Women with Severe Preeclampsia Compared to Normotensive, Healthy Pregnant Women

		Severely preeclamptic patients	Controls
PvuII	Genotype frequencies		
	Genotype	$n=119$ (100%)	$n=103$ (100%)
	TT	37 (31.1%)	32 (31.1%)
	TC	56 (47.1%)	53 (51.4%)
	CC	26 (21.8%)	18 (17.5%)
	$\chi^2=0.75$, df=2, $p>0.05$		
	Allele frequencies		
	T	130 (54.6%)	117 (56.8%)
	C	108 (45.4%)	89 (43.2%)
XbaI	Genotype frequencies		
	Genotype	$n=119$ (100%)	$n=103$ (100%)
	AA	38 (31.9%)	25 (24.3%)
	AG	62 (52.1%)	64 (62.1%)
	GG	19 (16.0%)	14 (13.6%)
	$\chi^2=2.33$, df=2, $p>0.05$		
	Allele frequencies		
	A	138 (58.0%)	114 (55.3%)
	G	100 (42.0%)	92 (44.7%)

extension at 72°C for 30 s, ending with a final extension at 72°C for 10 min and cooling to 4°C in a thermocycler (Model 2400, Perkin Elmer, Norwalk, USA). The PCR products, which contained a part of intron 1 of the ESR1 gene, were digested overnight with PvuII and XbaI restriction enzymes (Roche, Basel, Switzerland) at 37°C, producing fragments of 255 bp (C allele) or 97+158 bp (T allele) and of 255 bp (G allele) or 142+113 bp (A allele), respectively. The cleavage products were electrophoresed on 3% agarose gel and stained with ethidium bromide. The restriction fragments were detected with visual inspection on an ultraviolet table.

Statistical Analysis

The normality of continuous variables was tested using the Shapiro-Wilk *W*-test. We used the *t*-test to compare continuous parametric variables between the severely preeclamptic and control groups, whereas for continuous nonparametric variables we used the Mann-Whitney *U*-test. The Pearson χ^2 test was performed to compare categorical variables between groups. Multivariate logistic regression was carried out with adjustment for maternal age, prepregnancy body mass index (BMI), primiparity and smoking status.

An exact test using a Markov chain was applied to check whether the observed genotype frequencies were in Hardy-Weinberg equilibrium, which is more suitable for a small sample size than the χ^2 analysis. Linkage disequilibrium (LD) was assessed with a permutation test using the Expectation-Maximization (EM) algorithm. Our data were genotypic data with an unknown gametic phase. Therefore, we carried out haplotype estimation using a pseudo-Bayesian approach (Excoffier-Laval-Balding [ELB] algorithm).

Statistical analyses were performed using the following software: STATISTICA (version 6.1; StatSoft, Tulsa, USA) and SPSS (version 13.0 for Windows; SPSS, Chicago, USA). For population genetic data analysis, Arlequin software (version 3.0; CMPG, University of Berne, Switzerland) was applied. For all statistical analyses, $p<0.05$ was considered statistically significant.

Results

Patient Characteristics

The clinical characteristics of the severely preeclamptic and normotensive, healthy pregnant women are described in Table 1. The systolic and diastolic blood pressures were significantly higher in the severely preeclamptic group than in the control group. There were no statistically significant differences between the groups in maternal age or in the percentage of smokers. The frequency of primiparas and the prepregnancy BMI were significantly higher in the severely preeclamptic group. However, there was no significant difference in the percentage of prepregnancy obesity (prepregnancy BMI$\geq$30 kg/m^2) (data not shown). The gestational age at delivery was significantly lower in the severely preeclamptic group, which in part explains why fetal birth weight was also significantly lower in this group. In addition, the frequency of fetal growth restriction was significantly higher in the severely preeclamptic patients than in the control group.

Table 3. Frequencies of PvuII and XbaI Genotype Combinations of the ESR1 Gene in the Severely Preeclamptic and Control Groups

Genotype	Severely preeclamptic patients (n=119)	Controls (n=103)
TT/AA	29 (24.4%)*	10 (9.7%)
TT/AG	6 (5.0%)†	19 (18.4%)
TT/GG	2 (1.7%)	3 (2.9%)
TC/AA	8 (6.7%)	12 (11.7%)
TC/AG	48 (40.3%)	36 (35.0%)
TC/GG	0	5 (4.9%)
CC/AA	1 (0.9%)	3 (2.9%)
CC/AG	8 (6.7%)	9 (8.7%)
CC/GG	17 (14.3%)	6 (5.8%)
χ^2=29.05, df=8, p<0.001		

*p=0.003, adjusted odds ratio (OR): 4.36, 95% confidence interval (CI): 1.65–11.53, TT/AA *vs*. other genotypes. †p=0.002, adjusted OR: 0.17, 95% CI: 0.06–0.53, TT/AG *vs*. other genotypes. Adjustment was carried out for age, prepregnancy BMI, primiparity and smoking status. ESR1, estrogen receptor α; BMI, body mass index.

Table 4. T-A Haplotype Carriers and Noncarriers of the PvuII and XbaI ESR1 Gene Polymorphisms in the Severely Preeclamptic and Control Groups

	Severely preeclamptic patients (n=119)	Controls (n=103)
Homozygous T-A carriers	29 (24.4%)*	10 (9.7%)
Heterozygous T-A carriers	62 (52.1%)†	67 (65.1%)
Noncarriers	28 (23.5%)	26 (25.2%)

*Adjusted odds ratio (OR): 4.36, 95% confidence interval (CI): 1.65–11.53, homozygous T-A carriers *vs*. heterozygous T-A carriers + noncarriers, p=0.003. Adjusted OR: 5.64, 95% CI: 1.83–17.38, homozygous T-A carriers *vs*. noncarriers, p=0.003. †Adjusted OR: 1.34, 95% CI: 0.64–2.83, heterozygous T-A carriers *vs*. noncarriers, p>0.05. Adjustment was carried out for age, prepregnancy BMI, primiparity and smoking status. ESR1, estrogen receptor α; BMI, body mass index.

ESR1 Genotypes

The genotype and allele frequencies of the PvuII and XbaI ESR1 gene polymorphisms in the two groups are shown in Table 2. The PvuII and XbaI ESR1 genotype distributions were in Hardy-Weinberg equilibrium in both groups. There were no significant differences between the groups in the genotype and allele frequencies of PvuII and XbaI polymorphisms. However, regarding the simultaneous carriage of both polymorphisms (Table 3), the TT/AA genotype combination occurred significantly more frequently in the severely preeclamptic group (24.4% *vs*. 9.7%, p=0.003), whereas the frequency of the TT/AG genotype combination was significantly lower in that group (5.0% *vs*. 18.4%, p=0.002). Although the CC/GG genotype combination was more frequent in the severely preeclamptic patients, this association disappeared in a multivariate logistic regression model after adjustment for maternal age, prepregnancy BMI, primiparity and smoking status (data not shown).

There was a strong linkage disequilibrium between the PvuII and XbaI ESR1 gene polymorphisms. Indeed, the two polymorphisms are only 50 bp apart in the first intron of the ESR1 gene. According to the haplotype estimation, the homozygous T-A haplotype carriers had an increased risk of severe preeclampsia, independent of maternal age, prepregnancy BMI, primiparity and smoking status (adjusted odds ratio [OR]: 4.36, 95% confidence interval [CI]: 1.65–11.53, Table 4). The risk of heterozygous T-A haplotype carriers did not differ from that of noncarriers (adjusted OR: 1.34, 95% CI: 0.64–2.83). No association was observed between severe preeclampsia and the C-G, T-G and C-A haplotypes of the ESR1 PvuII and XbaI polymorphisms after adjustment for maternal age, prepregnancy BMI, primiparity and smoking status (data not shown).

We also investigated the association between fetal growth restriction and the maternal ESR1 genotypes in severely preeclamptic patients. Simultaneous carriage of the two polymorphisms and the PvuII polymorphism separately was not associated with fetal growth restriction in severely preeclamptic patients (data not shown). However, the GG genotype of the XbaI polymorphism was associated with a lower risk of fetal growth restriction in patients with severe preeclampsia (OR: 0.23, 95% CI: 0.07–0.73, Table 5).

The power of our study to detect the observed differences in the genotype distributions between the study groups, at a Type I error rate of 0.05, was greater than 80%.

Discussion

We here investigated the association between two intronic polymorphisms of the ESR1 gene and preeclampsia in a Caucasian population.

In a study of placental bed biopsy specimens, Malamitsi-Puchner *et al*. failed to find an association between preeclampsia and either a codon 10 or a codon 87 polymorphism in exon 1 of the ESR1 gene (*22*). Tempfer *et al*. reported that in a multigenic model, the combination of Factor V Leiden, NOS 3 T768C, NOS 3 Glu298Asp and ESR1 PvuII polymorphisms is associated with severe preeclampsia (*23*). Maruyama *et al*. observed that a single nucleotide polymorphism of the estrogen receptor β (ESR2) gene (rs928554) is linked to preeclampsia after stratification according to the presence of a family history of hypertension (*24*).

In our study, we found that homozygous T-A haplotype carriers of ESR1 PvuII and XbaI polymorphisms have an

Table 5. XbaI Genotype Frequencies of the ESR1 Gene in Severely Preeclamptic Mothers with and without Fetal Growth Restriction

	Genotype	Severely preeclamptic mothers	
		Without fetal growth restriction ($n=61$)	With fetal growth restriction ($n=58$)
XbaI	AA	15 (24.6%)	23 (39.7%)
	AG	31 (50.8%)	31 (53.4%)
	GG	15 (24.6%)	4 (6.9%)*
Statistical analysis	$\chi^2=7.98$, df=2, $p=0.018$		

*$p=0.011$, odds ratio (OR): 0.23, 95% confidence interval (CI): 0.07–0.73, GG *vs*. other genotypes. ESR1, estrogen receptor α.

increased risk of severe preeclampsia. Furthermore, the GG genotype of the ESR1 XbaI polymorphism was associated with a lower risk of fetal growth restriction in severely preeclamptic patients.

An intronic polymorphism could exert phenotypic effects through several mechanisms. It may enhance or reduce gene transcription, and it may also affect the splicing of RNA, producing alternatively spliced mRNA variants with resultant significant changes in gene function (*25–27*). In addition, an intronic polymorphism may also be linked to another, truly functional sequence variant and may therefore be a genetic marker of another polymorphism. In the case of the ESR1 PvuII gene polymorphism, it has been reported that the C allele, but not the T allele, forms part of a B-myb transcription factor binding site and functions as an intragenic enhancer (*28*). Interestingly, the transcription of myb is upregulated by estrogen (*29*). Therefore, the presence of the T allele may result in reduced ESR1 expression, and thus the ESR1-mediated effects of estrogen may be decreased, leading to a relative estrogen deficit. The XbaI polymorphism might also have a functional importance that remains unknown. Indeed, the XbaI polymorphism, but not the PvuII polymorphism, was associated with fetal growth restriction in severely preeclamptic patients, and the two polymorphisms had an effect on the risk of severe preeclampsia only in combination.

Estrogen has several ESR1-mediated effects—including both direct effects on vascular tissues and systemic effects—which may influence the risk for preeclampsia (*2*). Estrogen exerts vasodilatory effects through ESR1 in both a rapid, nongenomic manner and a genomic manner. ESR1 also mediates two other effects of estrogen in the vessel wall: acceleration of re-endothelialization after vascular injury (*30*) and inhibition of the vascular injury response. ESR1 and ESR2 are necessary and sufficient for estrogen-mediated protection against vascular injury (*31*, *32*). Furthermore, ESR1 also mediates systemic effects of estrogen, such as changes in lipid profile, alterations in coagulation and fibrinolytic systems and antioxidant effects (*2*).

We hypothesize that the relative estrogen deficit (reduced ESR1 expression) due to the sequence variations in the ESR1 gene leads to vasoconstriction in the systemic and uteroplacental circulation and, therefore, that the ESR1 gene polymorphisms may influence the risks of preeclampsia and fetal growth restriction.

ESR1 and ESR2 play important roles in the maintenance of uteroplacental and systemic circulation during pregnancy (*33*). Reduced ESR1 expression caused by a single nucleotide polymorphism of the ESR1 gene has already been implicated in the pathogenesis of spontaneous abortion (*34*). In addition, we previously reported that the ESR1 PvuII gene polymorphism is associated with perinatal morbidity in premature infants (*35*). In a non-primate model of preeclampsia, the spontaneous hypertension and heart failure (SHHF) rat, the placental expression of estrogen receptor–related protein (ICERE-1)—which correlates inversely with estrogen receptor expression—was upregulated (*36*). Furthermore, a woman with prior preeclampsia has a decreased risk of breast cancer, which is also consistent with reduced estrogen receptor expression in preeclamptic patients (*37*). Interestingly, the T-A haplotype of the ESR1 PvuII and XbaI gene polymorphisms was found to be associated with myocardial infarction and ischemic heart disease in postmenopausal women (*9*). Indeed, preeclampsia and cardiovascular disorders have many similar risk factors such as obesity, hyperlipidemia, as well as increased insulin resistance; and oxidative stress, inflammation, as well as endothelial injury are involved in the pathogenesis of both of them (*38*).

The limitation of our study is that it was designed as a case-control study. Although we made efforts to avoid population stratification—our study groups were homogenous and well matched in ethnicity—a transmission disequilibrium test (TDT) should be undertaken or multiple unlinked markers should have been used to completely preclude the possibility of this confounding effect.

In conclusion, homozygous T-A haplotype carriers of ESR1 PvuII and XbaI gene polymorphisms showed an increased risk of severe preeclampsia, which is independent of maternal age, prepregnancy BMI, primiparity and smoking status. In addition, the GG genotype of the ESR1 XbaI polymorphism decreased the risk for fetal growth restriction in severely preeclamptic patients. However, further studies are needed to explore the exact mechanism by which ESR1 gene polymorphisms influence the risks of preeclampsia and fetal growth restriction.

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