Genetic Polymorphism in Corticotropin-releasing Hormone Receptor Type-1 in Preeclamptic Korean Women

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Purpose: Placental corticotropin-releasing hormone receptor type 1 (CRHR1) expression is reduced in pregnancies with abnormal placental function such as preeclampsia (PE), and the levels and/or function of *CRHR1* are genetically influenced. The aim of this study was to investigate the association between the c.33+8199C>T polymorphism in the *CRHR1* gene and PE in a Korean population.

Materials and Methods: Using a case-control design, the association between the *CRHR1* polymorphism and the risk of PE was investigated in 203 individuals with PE and 211 normotensive controls. Genotypes were determined using a SNapShot kit and an ABI Prism 3100 Genetic analyzer.

Results: Genotypes and allele frequencies for the *CRHR1* polymorphism did not differ between PE and normotensive pregnancies. The variant T allele was more frequent than the ancestral C allele in both of the groups and was more frequent in the controls than in the cases. In risk analysis for PE, there was not an increased risk of preeclampsia in subjects who were concomitant homozygous rare allele genotypes (CC) (OR, 0.3; P=0.15) or heterozygous rare allele genotypes (TC) (OR, 0.8; P=0.29). There were no differences in the complications of PE such as severity or preterm delivery in patients with the *CRHR1* polymorphism.

Conclusion: Our findings indicate that the *CRHR1* polymorphism was not associated with PE in the present Korean study group.

Key Words: *CRHR1* polymorphism, Preeclampsia

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Introduction

Preeclampsia (PE), a leading cause of maternal and neonatal morbidity and mortality, is a multifactorial disease affecting about 5-10% of all pregnancies. It requires intense monitoring and clinical supervision[1)]. Pathogenic model of PE is known as aberrant feto-maternal immune interacion by abnormal

tissue and arterial invasion of trophoblasts[2, 3]. Therefore, the process of implantation between invasive trophoblast cells and the maternal endometerium is imporatnt in the maintenance of normal pregnancy[4]. However, the molecular interactions at the feto-maternal interface during the time of adhesion and subsequent invasion are not completely understood.

The hypothalamic corticotropin-releasing hormone (CRH) is produced in several organs of the female reproductive system, including the endometrial glands, decidualized stroma, and trophoblast[5-9]. CRH, locally produced in the female reproductive organs, promotes implantation and maintenance of early pregnancy[9]. Moreover, CRH plasma levels are higher in women who deliver preterm and are lower in women who deliver postterm, compared to those in women who deliver at term[10]. These differences are evident at as early as 10 weeks of gestation. It seems that the patterns of plasma CRH are associated with aberrant timing of delivery[10]. Moreover, CRH participates in the maintenance of vascular tone by activating a guanylate cyclase/nitric oxide pathway in the human placenta[11]. Therefore, CRH plays a major role in controlling the mechanisms for the maintenance of pregnancy.

All members of the CRH family exert their effect by binding to CRH receptors. To date, two CRH receptors have been identified, both of which belong to the class II G-protein-coupled receptors that are linked to a number of intracellular signaling pathways including adenylate cyclase-cAMP protein kinase A, mitogen-activated protein kinase, and protein kinase C[12]. These receptors share 70% identity at the amino acid level, but have different binding properties from those of the members of the CRH family.

The gene encoding the CRH receptor type 1 (*CRHR1*) is located on 17q12-q22 and is expressed in human endometrial and myometrial cells, indicating a local effect of uterine CRH[13, 14]. Especially, placental *CRHR1* expression is reduced in pregnancies with abnormal placental function such as PE[15], and the levels and/or function of *CRHR1* are genetically influenced. Especially, intron 1 of *CRHR1* contains highly conserved regions that may have regulatory functions (according to the UCSC Genome Browser database, http://genome.ucsc.edu/). Functional intronic regulatory element has been reported for several genes[16, 17]; this *CRHR1* intronic region could affect transcriptional modulation of gene function. The rs7209436 (c.33+8199C>T) is one among representative polymorphisms being in intron 1 of *CRHR1* gene. Forthermore, it has been recently reported to show strong interaction with development of adult depression following childhood maltreatment[18]. However, the impact of the *CRHR1* gene polymorphism on the risk of PE has not been investigated.

The aim of this study was to evaluate the allele and genotype frequencies of the *CRHR1* gene polymorphism in PE and normotensive pregnancies and to investigate whether there is an association between this polymorphism and PE.

Materials and Methods

1. Subjects

This study involved 414 pregnant third trimester women at Kwan Dong University Cheil General Hospital and at Ewha Women's University MokDong Hospital in Seoul, Korea. All participants included in this study were of Korean origin. Pregnant women that developed gestational hypertension without proteinuria and pregnant women with an abnormal fetal karyotype, chromosomal abnormalities, chronic hypertension, diabetes, or renal disease were excluded from this study. Appropriate institutional review board (IRB) approval from the Ethics Committee at Cheil General Hospital was obtained for this study.

The study population included women who developed PE (n=203) and healthy pregnant women (n=211) in their third trimesters. Normal pregnancy controls consisted of normotensive women who delivered a healthy neonate at term (>37 weeks of gestation) without significant medical or obstetric complications such as chronic hypertension, diabetes, renal insufficiency, congenital anomalies, or fetal demise. PE was defined as new onset of hypertension (systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg) and proteinuria (≥300 mg in a 24-hour urine collection and/or ≥ 2+ on dipstick testing) after 20 weeks of gestation. For analysis of the differences in complications

of PE, the PE group was divided into two subgroups: severe PE (n=133) and PE with preterm delivery (n=105). Severe PE was defined as preeclampsia with severe hypertension (DBP ≥110 mmHg) and/or severe proteinuria (urinary protein excretion ≥5 g per 24 hours and/or ≥3+ on dipstick testing). Preterm delivery was defined as the delivery of a neonate at fewer than 37 weeks of gestation.

2. DNA Extraction and Genotyping

Peripheral blood was collected from all participants into ethylenediaminetetraacetic acid tubes (Becton Dickinson, Franklin Lakes, USA). Immediately after the sampling, genomic DNA was extracted using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations and was stored at −80℃ until required for analysis.

The *CRHR1* polymorphism was analyzed using a single base primer extension assay performed with the SNapShot kit (Applied Biosystems, Foster City, USA) and an ABI Prism 3100 Genetic analyzer, according to our previous method[19, 20]. The polymorphism in the intron region of *CRHR1* is located at position 9144294 from the human genomic contig NT_010783.15 (NCBI build 37.1). A polymerase chain reaction (PCR) was used to amplify the genomic DNA region containing the polymorphism. The primers for PCR were as follows: forward: 5'- GAGCTTCTGCCCCCAAGTCT-3' and reverse: 5'- GGGCTCAGGTTAGATGTGGGT-3'. Primer extension reactions were performed with the SNapShot ddNTP Primer Extension Kit (Applied Biosystems, Foster City, USA) as recommended by the manufacturer. The extension primer was 5'- GGCTGTCCCACAACATGGGGTCTTACAG-3'. One microliter of the final reaction samples with extension products and GeneScan 120 Liz size-standard solution were added to 9 μL of Hi-Di formamide (Applied Biosystems, Foster City, USA). The mixtures were analyzed on a 3100 Avant genetic analyzer (Applied Biosystems, Foster City, USA) using Genotyper software version 3.7 (Applied Biosystems, Foster City, USA).

3. Statistical analysis

Data are presented as the mean±standard deviation (SD) or number (%). The differences in clinical characteristics between the two groups were analyzed using the χ^2-test and Student's t-test. The genotype frequencies of the polymorphisms were tested for deviation from the Hardy-Weinberg equilibrium (HWE) using the Chi-square test (http://oege.org/software/hardy-weinberg.shtml). Odds ratios (OR) and 95% confidence intervals (CI) were calculated to assess the disease risk conferred by *CRHR1* genotypes. $P<0.05$ was considered statistically significant. The statistical analysis was performed with the Statistical Package for Social Sciences version 12.0 (SPSS Inc., Chicago, USA).

Results

Polymorphism analysis of the *CRHR1* gene was performed in all subjects. The clinical characteristics of the study population are presented in Table 1. The maternal age and nulliparity were not different between the PE and control cases ($P>0.05$). However, the maximum systolic and diastolic blood pressures were significantly higher in PE than in the control cases ($P<0.001$). Proteinuria was detected only in cases of

Table 1. Clinical Characteristics of the Study Population

Characteristics	Control (n=211)	Preeclampsia (n=203)	*P* value
Maternal age (years)	31.4±4.3	30.7±4.0	0.409
Nulliparity (n)	125 (59.2%)	137 (67.5%)	0.084*
Maximum systolic blood pressure (mmHg)	117.2±13.5	157.9±17.5	<0.001
Maximum diastolic blood pressure (mmHg)	76.5±10.1	99.5±12.1	<0.001
Proteinuria (dipstick testing)	−	2.7±1.0	−
Gestational age at delivery (weeks)	39.1±1.7	35.9±4.0	<0.001
Fetal birth weight (g)	3,208.4±460.5	2,481.2±889.2	<0.001

*χ^2-test

PE. At delivery, gestational age and fetal birth weight were significantly lower in PE than in the control cases (P<0.001).

The genotypes and allele frequencies of the *CRHR1* polymorphism of normal controls and patients with PE are presented in Tables 2. The distributions of genotype frequencies in each group were in Hardy-Weinberg equilibrium (HWE) (controls: χ^2=2.81 and P=0.245, PE: χ^2=0.12 and P=0.942, respectively). Allelic and genotypic frequencies did not differ between the two groups (P>0.05 in both). The frequencies of the rare allele (C allele) and the homozygote rare genotype (CC) were higher in the control group than in the PE group, but the difference was not significant.

The ORs of the disease according to the *CRHR1* polymorphism are presented in Tables 3. No association was found between the *CRHR1* polymorphism and preeclampsia risk [OR (95% CI): 0.76 (0.46-1.26) for TC and 0.32 (0.06-1.63) for CC, respectively]. In both the recessive and dominant genotype model, the risks of PE in women with the homozygote rare genotype (CC) were more decreased in the recessive model [OR (95% CI): 0.34 (0.07-1.70] than in the dominant model [OR (95% CI): 0.70 (0.43-1.15)], but not significantly.

We divided the PE group into two subgroups based on disease complications. The homozygote rare genotype (CC) was not associated with the increased risks for the complications of PE [OR (95% CI) in severe PE and preterm PE: 0.25 (0.03-2.13) and 0.33 (0.04-2.78), respectively]. In both the recessive and dominant genotype model, the risks of complications of PE in women with the homozygote rare genotype (CC) were more decreased in the recessive model [OR (95 % CI): 0.30 (0.03-2.17) for severe PE and 0.33 (0.04-2.77) for preterm PE] than in the dominant model [OR (95% CI): 0.81 (0.47-1.39) for severe PE and 0.93 (0.52-1.64) for preterm PE], but there was not significant.

Table 2. Genotypes and Allele Frequencies of the *CRHR1* Polymorphism (c.33+8199C>T)

	Control n (%)	Preeclampsia n (%)	*P* value
Genotypic distribution			
CC	6 (2.9)	2 (1.0)	0.092
TC	41 (19.4)	32 (15.8)	
TT	164 (77.7)	169 (83.2)	
Allelic distribution			
C	53 (12.6)	36 (8.9)	0.093
T	369 (87.4)	370 (91.1)	

χ^2-test

Discussion

CRH, originally named corticotropin-releasing factor, is a polypeptide hormone involved in the stress response. CRH is a 41-amino acid peptide derived from a 191-amino acid preprohormone and is secreted by the paraventricular nucleus (PVN) of the hypothalamus in response to stress. CRH is also synthesized by the placenta and plays a role in the feto-

Table 3. Odds Ratios for Preeclampsia According to the *CRHR1* Polymorphism (c.33+8199C>T)

	Control n (%)	Preeclampsia			Severe Preeclampsia			Preterm Preeclampsia		
		n (%)	OR (95% CI)	*P* value	n (%)	OR (95% CI)	*P* value	n (%)	OR (95% CI)	*P* value
Genotype										
TT	164 (77.7)	169 (83.3)	1	-	108 (81.2)	1	-	83 (79.0)	1	-
TC	41 (19.4)	32 (15.8)	0.76 (0.46-1.26)	0.285	24 (18.0)	0.89 (0.51-1.56)	0.680	21 (20.0)	1.01 (0.56-1.82)	0.968
CC	6 (2.9)	2 (0.9)	0.32 (0.06-1.63)	0.150	1 (0.8)	0.25 (0.03-2.13)	0.173	1 (1.0)	0.33 (0.04-2.78)	0.284
Recessive model										
TT+TC	205 (97.2)	201 (99.0)	1	-	132 (99.2)	1	-	104 (99.0)	1	-
CC	6 (2.8)	2 (1.0)	0.34 (0.07-1.70)	0.170	1 0.8)	0.30 (0.03-2.17)	0.181	1 (1.0)	0.33 (0.04-2.77)	0.282
Dominant model										
TT	164 (77.7)	169 (83.3)	1	-	108 (81.2)	1	-	83 (79.0)	1	-
TC+CC	47 (22.3)	34 (16.7)	0.70(0.43-1.15)	0.157	25 (18.8)	0.81 (0.47-1.39)	0.440	22 (21.0)	0.93 (0.52-1.64)	0.789

Abbreviations: OR, odds ratio; CI, confidence interval

maternal interface in the time of invasion of trophoblast cells and participates in implantation and maintanence of early pregnancy[9]. Furthermore, it acts as a marker for determining the length of gestation and the timing of parturition and delivery[10, 11]. Current theory suggests three roles of CRH in parturition; 1) CRH is associated with an increased level of dehydroepiandrosterone (DHEA), which has a role in preparing and stimulating cervical contractions; 2) CRH produces an increase in prostaglandin availability in uteroplacental tissues, and prostaglandins activate cervical contractions; and 3) prior to parturition, CRH may have a role in the inhibition of cervical contractions by increasing cAMP levels in the myometrium. Therefore, CRH is known as a potent vasodilator of the human fetoplacental circulation and may act as a local regulator of placental vascular tone.

The CRHRs, necessary factors for CRH action, are G-protein-coupled receptors. It induces local effect of CRH in endometrial and myometrial cells in the uterine[13, 14]. Kateris et al. reported that significant reductions in the expression of *CRHR1* mRNA and in its protein levels had been detected in preeclamptic placentas[15]. They suggested that the dampening of CRH-induced vasodilation could be attributable to the loss of CRHRs in placentas from preeclamptic pregnancies [15]. However, the association between *CRHR* polymorphism and PE has not yet been confirmed in Korean.

In this study, we analyzed, for the first time, the distribution and interaction of the *CRHR1* polymorphism in Korean patients with preeclampsia. We found that the c.33+8199C>T polymorphism of *CRHR1* gene was not associated with the risk of PE. Moreover, the homozygous genotype with the ancestral C allele showed the lowest frequencies in both of the groups and was more frequent in the control cases than in the PE cases. However, these findings were not statistically significant.

This study indicates that the c.33+8199C>T polymorphism of *CRHR1* gene is not associated with PE in a Korean population. Therefore, we suggest that c.33+8199C>T polymorphism of the *CRHR1* gene is not associated with susceptibility to the development of preeclampsia in pregnant Korean women. However, we only included Korean patients in this study and had a relatively small sample size. Our results therefore need to be confirmed in further studies involving a larger cohort of patients from other ethnic groups.

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국문초록

목적: Corticotropin-releasing hormone receptor type 1 (CRHR1)은 자간전증과 같은 비정상적인 태반의 기능을 가지는 산모에서 감소되어 나타나며, 그것의 발현이나 기능은 유전적으로 영향을 받는다. 이번 연구의 목표는 한국인에서 *CRHR1* 유전자 다형성인 c.33+8199C>T과 자간전증 사이의 연관성을 조사하는 것이었다.

대상 및 방법: *CRHR1* 유전자 다형성은 SNapShot kit와 ABI Prism 3100 Genetic analyzer를 이용하여 203명의 자간전증 임산부와 211명의 정상 임산부에서 측정되었고, 유전자 다형성과 자간전증 위험도 사이의 연관성을 분석하였다.

결과: *CRHR1* 유전자 다형성의 유전자형과 대립유전자 빈도는 자간전증 임산부와 정상 임산부 사이에 다르지 않았다. 자간전증 발생 위험도는 분석된 유전자 다형성의 드문 대립 형질(C)을 지닌 이종접합 유전자형(TC)이나 동형접합 유전자형(CC)을 수반하는 그룹에서 증가되지 않았다. *CRHR1* 유전자의 동형접합 유전자형(CC)을 수반하는 그룹에서 중증 자간전증과 조기 자간전증과 같은 자간전증의 합병증 발병 위험에도 차이가 없었다.

결론: 이 연구는 *CRHR1* 유전자 다형성인 c.33+8199C>T가 한국인 임신부의 자간전증 발생과 연관이 없음을 나타낸다.

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