No association between endothelin-1 gene polymorphisms and preeclampsia in Korean population

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Purpose : Preeclampsia is a major cause of maternal and perinatal mortality and morbidity and is considered to be a multifactorial disorder involving a genetic predisposition and environmental factors. Endothelin-1 (ET-1) is a potent vasoconstrictor peptide, and alterations in the ET-1 system are thought to play a role in triggering the vasoconstriction seen with preeclampsia. The aim of this study was to examine the frequency of the 4 common single-nucleotide polymorphisms (SNPs) (c.1370T>G, c.137_139delinsA, c.3539+2T>C, and c.5665G>T) of the ET-1 gene in normotensive and preeclamptic pregnancies and to investigate whether these SNPs are associated with preeclampsia in pregnant Korean women.
Methods : We analyzed blood samples from 206 preeclamptic and 216 normotensive pregnancies using a commercially available SNapShot kit and an ABI Prism 3100 Genetic analyzer.
Results : There were no significant differences in genotype or allele frequencies of the 4 SNPs in the ET-1 gene between preeclamptic and normotensive pregnancies. The respective frequencies of the 3 haplotypes (TDTG, GDCT, and TICT; >10% haplotype frequency) were 61%, 13% and 13%, respectively, in preeclampsic pregnancies and 62%, 14% and 12%, respectively, in normotensive pregnancies. The frequencies of these haplotypes were similar for both groups. Using multiple logistic regression analysis, we did not observe an increase in the risk of preeclampsia for the 4 SNPs of the ET-1 gene under either a recessive or dominant model.
Conclusion : This study suggests that the 4 SNPs of the ET-1 gene are not associated with an increased risk for preeclampsia in pregnant Korean women.

Key words : Endothelin-1, Single-nucleotide polymorphism, Preeclampsia

Introduction

Preeclampsia affects 5 to 10% of all pregnancies and is characterized by the onset of hypertension and proteinuria after 20 weeks of gestation. Although it is a leading cause of maternal and perinatal mortality and morbidity, the pathogenesis of preeclampsia remains unclear.

A number of epidemiological studies have confirmed that preeclampsia has a maternal genetic component. Furthermore, very recent data strengthen the role of the maternal genetic component in transmitting specific genetic risk factors, which, in turn, enhance preeclampsia susceptibility[1].

The ET-1 gene is located on chromosome 6p23-24 and

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spans approximately 5.5 kb with 5 exons and 4 introns[2, 3]. Five polymorphisms were detected: (1) a T>G transversion at position 1,370 from the start of transcription[4]; (2) an A insertion (I)/deletion (D) in exon 1 at position 138[4-6]; (3) a G>A transition in intron 1 at position 1932[4]; (4) a T>C transition in intron 2 at position 3539[4]; and (5) a G>T transversion at position 5,665 affecting the 61th nucleotide of exon 5, which predicts a lysine-asparagine change at codon 198 (Lys198Asn)[4, 7, 8]. c.5665G>T polymorphism has been associated with increased resting blood pressure (BP) levels in several cohorts of middle-aged adults[4, 7]. In a sample of white 25 to 64 year olds, systolic (SBP) and diastolic blood pressure (DBP) were higher in carriers of the T allele compared with the G/G homozygotes, but only in those who were overweight (ie, BMI >25 kg/m^2)[4]. In a second cohort of British 25 to 74 year olds, subjects homozygous for the T allele had higher resting SBP and DBP levels than others, independent of obesity status[4]. Within a subsample that participated in a treadmill exercise task, carriers of the T allele who were obese exhibited the greatest increase in BP[4]. A recent study involving middle-aged Japanese subjects found that among obese individuals, carriers of the T allele exhibited higher DBP compared with noncarriers[7]. Barden et al.[9] found that T allele carrier status related to higher levels of both resting SBP and plasma ET-1, controlling for adiposity among women during pregnancy but not after birth. Moreover, concerning plasma ET-1 level, pregnant women with the T/T genotype have significantly elevated plasma ET-1 levels, and the T allele has been associated with raised SBP, suggesting the functional importance of this polymorphism in pregnancy[9]. However, the roles of the functional polymorphisms of the ET-1 gene have not yet to be discovered in preeclamptic patients.

Therefore, in this study we investigated the allele and genotype frequency of the 4 SNPs (c.1370T>G, c.137_139delinsA, c.3539+2T>C, and c.5665G>T) of the ET-1 gene in preeclamptic and nomortensive pregnancies and evaluated whether there was an association between these polymorphisms and preeclampsia.

Materials and Methods

1. Subjects

Participants were recruited when admitted for delivery at Cheil General Hospital and Ewha Womans University, MokDong Hospital in Seoul, Korean between 2003 and 2004. The study included 206 patients with preeclampsia and 216 maternal age- and sampling week- matched normal pregnancies. Written informed consent was obtained from all enrolled subjects before blood sampling, which was approved by the Ethics Committee of Cheil General Hospital and Ewha Womans University.

Preeclampsia was defined as the new onset of hypertension (SBP ≥140 mmHg or DBP ≥90 mmHg) and proteinuria (≥300 mg in a 24 hr urine collection or ≥1+ on dipstick testing of urine) after 20 weeks of gestation, according to the Committee Terminology of the American College of Obstetricians and Gynecologists (ACOG). Controls were selected randomly in the first trimester from contemporaneous women who were normotensive and who were without proteinuria throughout pregnancy, and who delivered a healthy neonate at term (>37 weeks of gestation) without significant medical or obstetric complications. Exclusion criteria included major chronic hypertension, diabetes, renal disease insufficiency, congenital anomalies, fetal chromosomal abnormalities, recurrent miscarriage, fetal growth retardation, abruptio placenta, or thrombophilia.

2. DNA Extraction and Genotyping

Peripheral blood was collected in EDTA vacutainer tubes (Becton Dickinson, CA USA) and stored immediately after collection at −80℃ until required for analysis. Genomic DNA was extracted form whole blood using a QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden Germany) according to the manufacturers recommendations (following the manufacturers protocol)[1].

The genotyping was analyzed by single base primer extension assay using the SNapShot kit according to manu-

factures recommendation (Applied Biosystems, CA, USA). Briefly, the genomic DNA region containing the 4 SNPs was amplified with PCR reaction. PCR was performed in a final volume of 10 uL containing 30 ng of genomic DNA, 1X PCR Buffer, 3 mM MgCl$_2$, 250 mM dNTPs, 1.25 pmoL of each primer, and 0.15 U of Taq DNA polymerase (Applied Biosystems, CA, USA). Amplification was carried out in a GeneAmp PCR System 9,700 thermal cycler (Applied Biosystems, CA, USA) under touch-down conditions (Don et al. 1991.) The primer sequences are shown in Table 1.

After amplification, the PCR products were purified through incubation with 1 U each of shrimp alkaline phosphatase (SAP) (Rhoche/Roche Diagnostic Systems, Norwalk, CT, USA) and exonuclease I (USB Corporation) at 37℃ for 1 hr and 72℃ for 15 min. Primer extension reactions were performed with the SNapShot ddNTP Primer Extension Kit (Applied Biosystems, CA, USA) as recommended by of the manufacturer. One microliter of the purified amplification products were added to a SNapShot Multiplex Ready reaction mixture containing 0.15 pmoL of genotyping internal primer. The primer extension reaction was carried out for 25 cycles for 96℃ for 10 s, 50℃ for 5 s, and 60℃ for 30 s. One microliter of the final reaction samples, plus extension products and GeneScan 120 Liz size-standard solution, were added to 9 ul of Hi-Di formamide (Applied Biosystems, CA, USA). The mixture was incubated at 95℃ for 5 min, followed by 5 min on ice, and then electrophoresed on an ABI Prism 3100 Genetic Analyzer. The results were analyzed by using the program of the ABI Prism GeneScan and Genotyper (Applied Biosystems, CA, USA).

3. Statistical analysis

Data are presented as the mean±SD or number (%). The clinical characteristics of case and control groups were compared with Students t-test and Chi-square test. The comparison of allele and genotype frequencies of the ET-1 polymorphisms between the two groups was performed with the Chi-square or Fishers exact test. HardyWeinberg equilibrium was tested in patients and controls, separately, by means of the Chi-Squared analysis (www.fourmilab.ch/rpkp/experiments/analysis/chiCalc.html) Haplotype frequencies for 4 SNPs of the ET-1 gene were estimated using program HapAnalyzer 1.0 version (http://hap.ngri.go.kr). Odds ratios (OR) and 95% confidence intervals (CI) were calculated to assess the disease risk conferred by genotypes. We also calculated the adjusted OR and 95% CI of each genotype and alternative models (recessive and dominant models) using logistic regression analysis. $P<0.05$ was considered statistically significant. The statistical analysis was performed with the Statistical Package for Social Sciences version 10.0 (SPSS Inc., Chicago, USA).

Results

Four SNPs analysis of the ET-1 gene was performed for 206 preeclamptic and 216 normotensive pregnancies. The clinical characteristics of the study population are shown in Table 2. Nulliparity, blood pressures, maternal weight at delivery, gestational age at delivery, birth weight, and BMI

Table 1. Nucleotide Sequences of the PCR and SNapShot Primers for Genotyping Analysis of the 4 ET-1 Polymorphisms

SNPs	RS No.	PCR primers	SNapShot primers
c.1370T>G	rs1800541	Forward: 5'-CCCTCCATCCCCAGAAAAAC-3' Reverse: 5'- ATTGGCTTTTTCCGCTAGTCC-3'	CAATGATGATCATAGGTCTTACTGGGCCACTGTGAGCGCT
c.137_139delinsA	rs10478694	Forward: 5'-CTCCTGCAGTCCCAGCTCTC-3' Reverse: 5'-CCGTTCAAACTGAACCCAAAG-3'	GCAGGCGCTGCCTTTTCTCCCCGTTAAA
c.3539 + 2T>C	rs1800543	Forward: 5'-GTCAGGGCCATTGATGCAC-3' Reverse: 5'-CCAAGTCCATACGGAACAACG-3'	ATAATAGGTGTGTCCATGTGTCATTTTAAAGACTATTAAT
c.5665G>T	rs5370	Forward: 5'-GGTCGGAGACCATGAGAAACA-3' Reverse: 5'-TGCTCCTGCTCTGATCCCA-3'	TGATATCTTTTCATGATCCCAAGCTGAAAGGCAA

ET-1, endothelin-1; SNPs, single-nucleotide polymorphisms; RS No., recommeded standard number

Table 2. Clinical Characteristics of Preeclampsia (PE) and Controls (CON)

Characteristics	PE (N=206)	CON (N=216)	*P* value*
Age (year)	31.0±4.5	31.3±4.2	0.384
Nulliparity (n)	146 (70.9)	131 (60.7)	0.035[†]
Maximum DBP (mm Hg)	99.8±12.5	76.1±10.0	⟨0.001
Maximum SBP (mm Hg)	158.2±18.0	116.8±13.3	⟨0.001
Maternal weight at delivery (kg)	72.3±11.2	67.9±9.2	⟨0.001
Birth weight (g)	2380.9±889.7	3202.0±458.8	⟨0.001
Body Mass Index (kg/m^2)	28.1±4.1	26.4±3.5	⟨0.001
Delivery weeks (wks)	35.7±3.9	39.1±1.7	⟨0.001
Proteinuria (dipstick)	2.8±1.0	–	–

DBP, diastolic blood pressure; SBP, systolic blood pressure; N, Number
Data were expressed as the mean±SD or number (%).
A *P* value was calculated using the *Student's t-test or [†] Chi-Squared test.
Significant value was taken at the level of *P*<0.05.

Table 3. Genotype and Allele frequencies of the 4 ET-1 Polymorphisms in Preeclampsia (PE) and Controls (CON)

SNPs		PE	CON	*P* value
c.1370T⟩G	Genotype			
	T/T	129 (62.6)	136 (63.0)	–
	T/G	68 (33.0)	71 (32.9)	0.963
	G/G	9 (4.4)	9 (4.2)	0.914
	Allele			
	T	326 (79.1)	343 (79.4)	–
	G	86 (20.9)	89 (20.6)	0.922
c.137_139delinsA	Genotype			
	Del/Del	138 (67.0)	153 (70.83)	–
	Del/Ins	66 (32.0)	56 (25.93)	0.216
	Ins/Ins	2 (1.0)	7 (3.24)	0.182
	Allele			
	Del	342 (83.0)	362 (83.8)	–
	Ins	70 (17.0)	70 (16.2)	0.759
c.3539+2T⟩C	Genotype			
	T/T	108 (52.4)	110 (50.9)	–
	T/C	82 (39.8)	91 (42.1)	0.674
	C/C	16 (7.8)	15 (6.9)	0.829
	Allele			
	T	298 (72.3)	311 (72.0)	–
	C	114 (27.7)	121 (28.0)	0.912
c.5665G⟩T	Genotype			
	G/G	110 (53.4)	112 (51.9)	–
	G/T	80 (38.8)	89 (41.2)	0.665
	T/T	16 (7.8)	15 (6.9)	0.830
	Allele			
	G	300 (72.8)	313 (72.5)	–
	T	112 (27.2)	119 (27.5)	0.906

SNPs, single nucleotide polymorphisms; ET-1, endothelin-1
Data were expressed as number (%).
Significant value was taken at the level of *P*<0.05.

were found to be significantly different between preeclamptic and nomortensive pregnancies (*P*<0.05). In contrast, there were no significant differences in the maternal age and birth height of the infants between the two groups (*P*>0.05).

The genotype and allele frequencies of the 4 SNPs (c.1370T>G, c.137_139delinsA, c.3539+2T>C, and c.5665G>T) in the ET-1 gene are listed in Table 3. The genotype frequencies in preeclamptic patients and normal controls did not significantly deviate from Hardy-Weinberg equilibrium (*P*>0.05). No significant difference between preeclamptic and normal pregnancies was observed in the genotype and allele frequencies of the 4 ET-1 polymorphisms (*P*>0.05, Table 3). The respective frequencies of the 3 haplotypes (TDTG, GDCT, and TICT; >10% haplotype frequency) were 61%, 13%, and 13% in preeclampsia and were 62%, 14%, and 12% in controls (Table 4). The frequencies of these haplotypes were similar in both the two groups (*P*>0.05).

Table 5 show the unadjusted and adjusted OR and 95% CI from multiple logistic regression analysis for the association between genotypes and preeclampsia risk according to recessive and dominant models, respectively. We adjusted for maternal age, nulliparity, delivery week, and BMI. There were no significant differences in the 4 SNPs of the ET-1 gene and preeclampsia risk under recessive and dominant models (*P*>0.05, Table 5).

Table 4. Haplotype frequencies of the 4 ET-1 Polymorphisms in Preeclampsia (PE) and Controls (CON)

Haplotype	c.1370T>G	c.137_139delinsA	c.3539+2T>C	c.5665G>T	PE (N=206)	CON (N=216)
1	T	D	T	G	61	62
2	G	D	C	T	13	14
3	T	I	C	T	13	12
4	G	D	T	G	8	6
5	T	I	T	G	3.2	4.4
6	T	D	C	T	1.2	1.0
7	T	I	C	G	0.3	0.4
8	G	I	C	G	0.1	0.1
9	G	D	C	G	0.2	0.1

D, del; I, insl; N, number; ET-1, endothelin-1
Data were expressed as percentage.

Table 5. Genotype Analysis in Recessive and Dominant Models

SNPs	Recessive		Dominant	
	Unadjusted OR (95% CI)*	Adjusted OR (95% CI)*	Unadjusted OR (95% CI)*	Adjusted OR (95% CI)*
c.1370T>G	1.1 (0.41-2.70)	1.2 (0.41-3.43)	1.0 (0.68-1.51)	1.0 (0.59-1.58)
c.137_139delinsA	0.3 (0.06-1.43)	0.8 (0.11-5.13)	1.2 (0.79-1.81)	1.3 (0.74-2.08)
c.3539+2T>C	1.1 (0.54-2.35)	1.3 (0.54-3.35)	0.9 (0.64-1.38)	0.9 (0.57-1.48)
c.5665G>T	1.1 (0.54-2.35)	1.3 (0.54-3.35)	0.9 (0.64-1.38)	0.9 (0.55-1.44)

OR, odds ratios; CI, confidence interval; SNP2, single nucleotide polymorphisms
*Adjusted for maternal age, nulliparity, delivery week, and BMI

Discussion

ET-1 is the most powerful endogenous vasoconstrictor peptide known to date[3] and able to influence placental blood flow either directly or in concert with other vasoactive agents[10-12]. Alteration in the ET-1 vasoconstrictor system that could constitute the biological mechanism leading to the reduced placental blood flow and increased resistance to flow in the feto-maternal circulation, which are characteristics of the pathophysiology of preeclampsia. In fact, plasma ET-1 levels have been shown to be increased in pregnancies complicated with preeclampsia[13].

Because of the role of ET-1 in vascular pathophysiology, the gene coding the ET-1 is an obvious candidate gene for coronary heart disease and essential hypertension. Previously, Dong et al.[14] reported on the association between lower SBP and some common ET-1 genotypes, alleles, and haplotypes in European American and black males. SNP analysis showed a borderline significant SBP-lowering effect of the 138I allele and an SBP-lowering effect of the 3539C allele for males only, accounting for 1.6% between-subject variation of SBP[14]. Haplotype analysis revealed that SBP growth curve levels in males homozygous for the 138D/3539C haplotype was 3.3 mm Hg lower than those homozygous for the 138D/3539T haplotype[14]. SBP in males homozygous for the haplotype (138I/3539C) combining the 2 BP-lowering alleles was 11.8 mm Hg lower than those homozygous for the most common haplotype[14]. However, this difference did not reach a statistical significance because of the low frequency of this 138I/3539C haplotype[14]. In our study, the genotype and allele frequencies of the 3 ET-1 SNPs (c.1370T>G, c.137_139delinsA, and c.3539+2T>C) were similar to those previously reported in a study of Dong et al.[14], but there was no difference between the frequency of the genotypes or alleles in preeclampsia compared with normal pregnancy. There have been no reports available on the c.1370T>G, c.137_139delinsA, and c.3539+2T>C polymorphisms of the ET-1 gene in preeclampsia,

which we studied here, yet. We also found that no increase in the risk of preeclampsia for the ET-1 gene was observed in both recessive and dominant models.

Several reports have described that the c.5665G>T polymorphism in ET-1 showed a positive association with BP elevation in overweight people, and this was observed not only in Caucasians but also in Japanese[4, 7, 8]. These studies suggested that the c.5665G>T polymorphism may affect BP regulation through the production of ET-1. The present study was investigated whether the ET-1 c.5665G>T polymorphism is associated with the risk of preeclampsia. The frequency of G/G, G/T, and T/T genotypes of this polymorphism in preeclampsia was 53.4%, 38.8%, and 7.8%, respectively, in our study, and this frequency was similar to that found in preeclampsia in the Australia population (52.8%, 41.7%, and 5.5%, respectively)[9]. Furthermore, in preeclamptic patients, the frequency of G and T alleles in the ET-1 c.5665G>T polymorphism was 72.5% and 27.5%, respectively, in our study, the Australia frequencies were 73.6% for G allele and 26.4% for T allele[9]. However, there were no significant differences in genotype or allele frequencies of the ET-1 c.5665G>T polymorphism between preeclamptic patients and controls. Interestingly, T allele carrier status related to higher levels of both resting SBP and plasma ET-1, controlling for adiposity among women during pregnancy but not after birth[9]. They indicated that the association of the T allele with raised BP and the T/T genotype with increased plasma ET-1 level suggest that this polymorphism may interact with other genes or environmental factors to sensitize pregnant women to develop preeclampsia[9].

Most common genetic disorders, such as preeclampsia, follow a complex inheritance and may result from variants of many genes, each contributing only a weak effect to the disease. Common genetic polymorphism may explain a portion of the heritable risk for common diseases. Consequently considerable effort should be devoted to finding and typing common SNPs in the human genome in order to understand the occurrence of relatively common phenotypes. Although we do not deny that one of the possible limitations of this case-control study is the relatively small sample size, we did take into account several issues that could lead to a false conclusion, such as established criteria for the diagnosis in order to exclude subphenotypes known to differ in the evaluation of the disease[15] and matching of cases and controls for several risk factors and for genetic background.

To our knowledge, this is the first study investigating the association between the 4 ET-1 SNPs (c.1370T>G, c.137_139delinsA, c.3539+2T>C, and c.5665G>T) and Korean patients with preeclampsia. In this study, the genotype, allele, and haplotype frequencies of these polymorphisms did not differ significantly between preeclamptic patients and healthy controls. We suggest that the ET-1 polymorphisms are not associated with susceptibility to the development of preeclampsia in Korean pregnant women. Our result might be indicative of the large diversity in the genetic background of preeclampsia, although this finding will need to be confirmed in a larger cohort of preeclamptic patients.

Acknowledgments

한글요약

목 적 : 자간전증은 임신부와 신생아의 사망 및 이환의 주된 원인으로 유전적 소질과 환경적 요인에 의해 발생하는 다요인성 질환이다. ET-1은 강력한 혈관수축 펩티드로 ET-1 시스템 내의 변경이 자간전증에서의 혈관수축을 자극하는 것으로 생각되고 있다. 본 연구에서는 정상 임신부와 자간전증 임신부에서 ET-1 유전자의 4가지 단일염기다형성들(c.1370T>G, c.137_139delinsA, c.3539+2T>C, and c.5665G >T)의 양상을 조사하여 비교함으로써 이러한 유전자 다형성들과 한국인 자간전증의 연관성에 대하여 알아보고자 하였다.

방 법 : 자간전증 임신부 206명과 임신기간 동안 자간전증이 발생하지 않은 정상 임신부 216명의 혈액으로부터 DNA를 추출하고 ET-1 유전자 다형성들의 양상을 SNapShot kit와 ABI Prism 3100 Genetic analyzer를 사용하여 분석하였다.

결 과 : 자간전증 환자군에서 ET-1 유전자의 4가지 단일염기다형성 각각의 유전자형 및 대립유전자의 빈도는 대조군과 유의한 차이가 없었다. 또한 자간전증 환자군에서 3가지 일배체형(TDTG, GDCT, and TICT)의 빈도도 대조군과 유의한 차이가 없었다: 61%(TDTG), 13%(GDCT), 13%(TICT) vs. 62%, 14%, 12%. 나이, 미산부율, 분만주수, 신체질량지수 등의 자간전증 발생요인을 공변량으로 하여 유전자형과 자간전증 발생의 위험도 사이의 연관성을 확인하기 위해 다중회귀분석을 시행한 결과 열성모델과 우성모델에서 모두 ET-1 유전자의 단일염기다형성들에 대한 자간전증 발생의 위험도가 증가하지 않았다.

결 론 : 한국인 임신부에서 ET-1 유전자의 4가지 단일염기다형성들은 자간전증 발생과 연관이 없는 것으로 사료된다.

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