Association between genetic polymorphisms in androgen receptor gene and the risk of preeclampsia in Korean women

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Abstract

Purpose To investigate associations between the androgen receptor (AR) polymorphisms as CAG repeats, GGC repeats and c.211G>A polymorphism and the risk of preeclampsia.

Methods The AR polymorphisms were experienced in 184 preeclamptic patients and 190 normal pregnancies and analyzed by multiple logistic regression.

Results Women with GGC repeats>16 were more frequently observed in preeclampsia, compared to those with GGC repeats≤16 [adjOR (95% CI): 3.64 (1.71–6.23)]. However, no significant differences were observed between the two groups with respect to CAG repeats. The genotypic and allelic frequencies of c.211G>A variant were significantly higher in cases than in controls ($P<0.05$ for both). In the combined distribution of these polymorphisms, the highest risk of preeclampsia was found among women with the haplotype as CAG>20/GA/GGC>16 [adjOR (95% CI): 4.26 (1.92–12.23)].

Capsule Longer GGC repeats and c.211G>A variant in the androgen receptor gene are associated with increased susceptibility to the risk of preeclampsia.

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Conclusions Our findings suggest that longer GGC repeats and c.211G>A variant in the AR gene are associated with increased susceptibility to the risk of preeclampsia.

Keywords Androgen receptor · CAG repeats · GGC repeats · c.211G>A polymorphism · Preeclampsia

Introduction

Preeclampsia, affecting about 5% of pregnancies, is characterized by hypertension and proteinuria after 20 weeks of gestation and results in substantial maternal and neonatal morbidity and mortality [1]. Although the underlying mechanism of preeclampsia has been investigated intensively for several years, the events leading to these alterations still remain unclear.

Steroid hormones of placental and fetal adrenal origin are importantly involved in the maintenance of pregnancy and development of the fetus [2]. An imbalance in the levels or activity of steroid hormones is associated with the development of gestational diseases including gestational hypertension, gestational diabetes, fetal growth restriction, and preeclampsia [3–5]. Especially, high androgen levels, primarily dependent on placental function, are known as a factor involved in the etiopathogenesis of preeclampsia [5–7]. Recently, an increased mRNA expression of the androgen receptor (AR) was founded in preeclamptic placentas and suggested that it may be associated with the development of preeclampsia via alteration of AR-mediated function on syncytiotrophoblasts and stromal cells in preeclamptic placentas [8]. These investigations indicate that the androgen and its receptor could contribute to the development of preeclampsia.

The AR is a real effector which is activating the transcription of androgen response gene through binding

with the androgen [9]. The *AR* gene is located between q11 and q12 on the X chromosome and consisted with eight exons [10]. Among genetic polymorphisms in the *AR* gene, CAG and GGC repeats in exon 1 of the *AR* gene have been known as the most common functional polymorphisms. CAG and GGC repeats result in variable lengths of polyglutamine and polyglycine tracts in transcriptional activation domain of the AR. Expansion and reduction of these repeats decrease not only activity of the AR but also expression of the AR mRNA and protein [11–13]. In addition, one single nucleotide polymorphism (SNP) is caused by a single G to A base change at codon 211 of the *AR* (c.211G>A). This substitution creates a recognition site for the restriction enzyme *Stu* I [14]. This SNP is located between CAG and GGC repeats, and has partial disequilibrium of CAG and GGC repeats [14, 15]. Therefore, these polymorphisms have studied as genetic factors in various disorders which are related to abnormal function of the AR, but are still poorly understood in preeclampsia. The aim of this study thus was to investigate whether these genetic polymorphisms of the *AR* gene are associated with susceptibility to preeclampsia in Korean women.

Materials and methods

Subjects

Subjects were recruited from the Obstetrics and Gynecology Department at Cheil General Hospital, between October 2001 and December 2004. The study subjects consisted of 180 women who developed preeclampsia during their pregnancy and 194 healthy pregnant. All subjects had no history of preexisting hypertension, diabetes mellitus, liver disease, or chronic kidney disease. Appropriate IRB approval of the Ethics Committee at Cheil General Hospital and written informed consent was obtained for this study.

Normal pregnancy ($n=190$) was defined as a woman who delivered healthy neonate at term (37 weeks of gestation or more) without medical or obstetric complications and was selected to the control for this study. Cases ($n=184$) were defined as women who developed preeclampsia during their pregnancy. Preeclampsia, severe preeclampsia, small for-gestational-age (SGA) and preterm delivery were defined as our previous studies [16, 17]; briefly, preeclampsia was defined as development of hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg) and proteinuria (≥ 300 mg in a 24 h urine collection and/or $\geq 1+$ on dipstick testing) after 20 weeks of gestation. Severe preeclampsia was defined as severe hypertension (diastolic blood pressure ≥ 110 mm Hg), severe proteinuria (urinary

protein excretion ≥ 5 g per 24 h and/or $\geq 3+$ on dipstick testing), thrombocytopenia (platelet count $<100\,000/\text{mL}$), severe central nervous system symptoms, or evidence of pulmonary edema and oliguria. SGA were defined as a birth weight below the 10th percentile according to the national birth weight distribution of a Korean population. Preterm delivery was defined as the delivery of a neonate at fewer than 37 weeks of gestation.

Genotyping of the AR genetic polymorphisms

Genomic DNA was extracted with DNA extraction kits (Qiagen, CA) from the peripheral blood of all subjects. CAG repeats and GGC repeats were analyzed using the quantitative fluorescence-polymerase chain reaction (QF-PCR) and c.211G>A polymorphism was genotyped using PCR-restriction fragment length polymorphism (PCR-RFLP).

The primers for PCR were as follows: for CAG repeats—forward: 5'-FAM-TCCAGAATCTGTTCCAGAGCGTGC-3', reverse: 5'-GCTGTGAAGGTTGCTGTTCTCAT-3'; for GGC repeats—forward: 5'-FAM-TCCTGGCACACTCTCTCTTAC-3', reverse: 5'-GCCAGGGTACCACACATCAGGT-3'; for c.211G>A polymorphism—forward: 5'-CACAGGCTACCTGGTCCTGG-3' and reverse: 5'-CTGCCTTACACAACTCCTTGG C-3'.

To determine the number of CAG repeats, the PCR reaction solution contained 10 ng genomic DNA, 10 pM primers, 0.25 mM dNTPs, 1.5 mM MgCl_2 , 1X buffer, and 0.25U Taq polymerase per 5 μl of total reaction volume. To determine the number of GGC repeats, the PCR reaction solution contained 10 ng genomic DNA, 10 pM primers, 0.25 mM dNTPs (containing deaza-7-GTP, Roche diagnostics, Germany), 1.5 mM MgCl_2 , GC rich PCR system (Roche diagnostics, Germany) per 5 μl of total reaction volume. PCR conditions included predenaturation at 95°C for 5 min, 35 cycles of 95°C for 30 s, 56°C for 30 s, 72°C for 60 s, and final extension at 72°C for 10 min. After PCR amplification, the FAM labeled DNA fragments were analyzed on an Applied Biosystems 3100 Avant genetic analyzer (Applied Biosystems Inc., CA) using the Pop-4 polymer and GeneScan-500 ROX size standard (Applied Biosystems Inc., CA). The number of CAG and GGC repeats was analyzed using the Genotyper 3.7 software. To determine the distribution of CAG and GGC repeats, we used the mean value of the two alleles (biallelic averages) because two X-linked *AR* alleles are present in women.

To determine the genotype of c.211G>A polymorphism, DNA (10 ng) was added to the reaction mixture (10 μL) containing 1X PCR reaction buffer, 1.5 mM MgCl_2 , 0.25 mM of each dNTP, 1 pM of primer pair, and 0.5 U Taq polymerase. Cycling conditions were 94°C for 5 min, 94°C for 30 s, 62°C for 30 s and 72°C for 30 s for 32

Table 1 Clinical characteristics of the study subjects

Characteristics	Control (<i>n</i> =190)	Preeclampsia (<i>n</i> =184)	<i>P</i> -value
Maternal age (years)	32.9±4.5	31.2±3.9	0.301
BMI (kg/m ²)	21.4±3.1	22.4±2.7	0.452
Nulliparity (<i>n</i>)	121 (63.7%)	154 (83.7%)	<0.001
Maximum systolic BP (mmHg)	121.9±7.2	159.5±16.5	<0.001
Maximum diastolic BP (mmHg)	75.4±8.7	101.5±11.9	<0.001
gestational age at delivery (weeks)	39.2±1.2	36.4±3.3	<0.001
Fetal birth weight (g)	3339.9±495.8	2569.6±806.4	<0.001
SGA infants (<i>n</i>)	0 (0%)	47 (25.5%)	–
Proteinuria (mg/24 hr)	–	3285.1±1021.4	–

BMI Body mass index; *BP* Blood pressure; *SGA* Small-for-gestational-age
Data are presented as mean±standard deviation for continuous variables and as number (percentage) for categorical variables.

cycles, and 72°C for 5 min. The PCR product size was 416 base pairs (bp). Stu I restriction enzyme was used to digest the site of the G allele of codon 211 into fragments of 329 and 87 bp. Final products were electrophoresed on a 3% nusieve agarose gel containing ethidium bromide and visualized using a Molecular Imager FX (Bio-Rad Laboratories Pty Ltd., USA).

Statistical analysis

Data were presented as the mean ± standard deviation (S.D.) or number (%). The differences in frequency or average of each characteristic between the two groups were analyzed by the Fisher's exact test and Student's *t*-test. Frequencies of the *AR* polymorphisms were calculated in each group including control, preeclampsia, preterm preeclampsia, severe preeclampsia, and SGA preeclampsia. Deviation from Hardy-Weinberg equilibrium (HWE) was tested by χ^2 -test. Multiple logistic regression analysis was used to estimate the value of these polymorphisms as risk factors of preeclampsia, controlling for potential confounding factors. Potential confounding factors included maternal age,

parity, body mass index (BMI), and gestational age at delivery. Adjusted odds ratios (adjORs) and their 95% confidence intervals (CIs) were calculated. In all tests, $P<0.05$ was considered statistically significant. Analysis was performed using SPSS 12.0 statistical software (SPSS Inc., USA). The statistical power of this study was calculated as our previous studies [16, 17]. The sample size in our study revealed >90% power to detect the anticipated differences between the case and control groups at an α error of 0.05.

Results

The clinical characteristics for cases and controls are shown in Table 1. Maternal age and BMI was not significantly different between the two groups. The systolic and diastolic blood pressures, and nulliparity were significantly higher in cases than in controls ($p<0.001$ for all). In contrast, at delivery, gestational age and fetal birth weight were significantly lower in cases than in controls ($p<0.001$ in both). SGA infants and proteinuria was detected only in cases.

Table 2 Distribution of the androgen receptor polymorphisms in controls and preeclampsia cases

	Control		Preeclampsia		<i>P</i> -value
	<i>n</i>	(%)	<i>n</i>	(%)	
CAG repeat					
Median (range)	20	(13–26)	20	(13–29)	0.357
GGC repeat					
Median (range)	16	(11–18)	16	(12–22)	0.279
c.211 G>A genotype					
GG	162	85.3	137	74.4	reference
GA	28	14.7	41	22.3	0.032
AA	0	0	6	3.3	–
GA/AA	28	14.7	47	25.6	0.010
c.211 G>A allelic distribution					
G	352	92.4	315	85.6	reference
A	28	7.6	53	14.4	0.003

Table 3 Adjusted odds ratios for preeclampsia according to the androgen receptor gene polymorphisms

n (%)	CAG repeat			GGC repeat			c.211 G>A		
	≤20	>20	OR [95% CI] ^a	≤16	>16	OR [95% CI] ^a	GG	GA/AA	OR [95% CI] ^a
Control	130 (68.4)	60 (31.6)	reference	176 (92.6)	14 (7.4)	reference	162 (85.3)	28 (14.7)	reference
Preeclampsia	122 (66.3)	62 (33.7)	1.04 [0.62–1.78]	148 (80.4)	36 (19.6)	3.64 [1.71–6.23]	137 (74.4)	47 (25.6)	1.87 [1.14–4.25]
Preterm Preeclampsia	50 (65.8)	26 (34.2)	1.11 [0.63–1.94]	56 (73.7)	20 (26.3)	5.12 [2.84–10.59]	55 (72.4)	21 (27.6)	2.89 [1.76–5.89]
Severe Preeclampsia	67 (65.7)	35 (34.3)	1.52 [0.92–2.50]	71 (69.6)	31 (30.4)	6.14 [2.41–17.12]	74 (72.5)	28 (27.5)	3.12 [1.52–7.05]
SGA Preeclampsia	28 (59.6)	19 (40.4)	1.75 [0.91–3.36]	34 (72.3)	13 (27.7)	5.42 [3.58–10.75]	37 (78.7)	10 (21.3)	1.86 [1.27–3.61]

OR Adjusted odds ratio; CI Confidence interval; SGA Small for-gestational-age.

^a Adjusted for maternal age, gravid, parity, and gestational age at delivery.

Referent was used the control group.

Table 2 presents the frequencies of the studied polymorphisms in cases and controls. The median value and range of CAG and GGC repeats were not different between the two groups [median (range) of CAG and GGC repeats: 20 (13–26) and 16 (11–18) in control; 20 (13–29) and 16 (12–22) in preeclampsia]. The genotype frequencies of the c.211G>A did not deviate significantly from the HWE in either group ($P=0.273$ in cases and $P=0.192$ in controls, respectively). The frequencies of GA genotype and combined GA/AA genotype for c.211 G>A were significantly higher in cases than in controls (GA: 22.3% versus 14.7%, $P=0.032$; GA/AA: 25.6% versus 14.7%, $P=0.010$) and homozygous variant genotype (AA) was observed only in the cases. The variant A allele frequency was also significantly higher in cases compared with that in controls (14.4% versus 7.6%, $P=0.003$).

The distributions of the studied polymorphisms in the control, preeclampsia, preterm preeclampsia, severe preeclampsia, and SGA preeclampsia groups and the adjORs associated with preeclampsia are shown in Table 3. In order to assess the risk associated with CAG and GGC repeats, the study subjects were dichotomized based on the median value of each repeats as prior studies [17–19]. Frequencies of CAG repeats>20 were not different between cases and controls (31.6% versus 33.7%, $P=0.741$); whereas GGC repeats>16 occurred more frequently in cases than in controls (7.4% versus 19.6%, $P=0.001$) and increased the risk of preeclampsia [adjOR (95% CI): 3.64 (1.71–6.23)]. Moreover, women with GGC repeats>16 were at higher risks of developing severe preeclamptic complications including preterm preeclampsia [adjOR (95% CI): 5.12 (2.84–10.59)], severe preeclampsia [adjOR (95% CI): 6.14 (2.41–17.12)], and SGA preeclampsia [adjOR (95% CI): 5.42 (3.58–10.75)]. In analysis for c.211 G>A, women with GA/AA genotype carrying variant allele displayed increased risks of developing preeclampsia, preterm preeclampsia, severe preeclampsia, and SGA preeclampsia compared to those in women with GG genotype [adjOR (95% CI): 1.87 (1.14–4.25), 2.89 (1.76–5.89), 3.12 (1.52–7.05), and 1.86 (1.27–3.61), respectively].

In addition, we evaluated the combined effect of these polymorphisms on preeclampsia (Table 4). When CAG repeats, GGC repeats and the c.211G>A were considered in the risk assessment of preeclampsia, the haplotype of CAG>20/GA/GGC>16 were more frequent in cases than in controls ($p<0.001$) and the highest risk of preeclampsia was detected among women with this haplotype [adjOR (95% CI): 4.26 (1.92–10.21)]. Moreover, the haplotype of CAG>20/AA/GGC>16 was observed only in cases.

Discussion

The etiology of preeclampsia originates in the placenta and, in particular, the trophoblast cells that are found only in this

Table 4 Combined haplotypes of the androgen receptor gene polymorphisms and the risk for preeclampsia

Haplotypes	Control (<i>n</i> =190)	Preeclampsia (<i>n</i> =184)	OR [95% CI] ^a
CAG ≤20/GG/GGC≤16	119 (62.6)	104 (56.5)	reference
CAG >20/GA/GGC>16	7 (3.7)	24 (13.0)	4.26 [1.92–12.23]
CAG >20/AA/GGC>16	0	4	–

OR Adjusted odds ratio; CI Confidence interval

^a Adjusted for maternal age, gravid, parity, and gestational age at delivery.

tissue [1]. Many factors of placental origin seen in the maternal circulation during healthy pregnancy are increased in preeclampsia. These include several inflammatory cytokines, steroids and free-radical species; all are associated with stimulation of the vascular disease. Especially, androgen is associated with vascular endothelial damage via reduction of DNA synthesis and enhancement of apoptosis on vascular endothelial cells [18]. And it stimulates the androgen-mediated gene expression binding to the AR, a type of nuclear receptor [9]. These AR-mediated actions play important roles in regulating physiological events essential to the maintenance of pregnancy and development of the placenta. Prior studies reported that levels of the androgen were significantly higher in women with preeclampsia than in normotensive women and this difference may indicate a role for androgen in the pathogenesis of preeclampsia [5–7]. Moreover, AR mRNA also increased in preeclamptic placentas compared with normal placentas [8]. These investigations demonstrate that abnormal expression in androgen and its receptor may be a possible mechanism for their association with preeclampsia.

The transcriptional activity of the AR is influenced by the polyglutamine and polyglycine tracts that are produced by the highly polymorphic CAG and GGC repeats in exon 1 of the *AR* gene [10]. Expansion of the CAG repeat decreases not only transcriptional activity of the AR but also expression of the AR mRNA and protein [11, 12]. Expansion of the GGC repeat also decreases AR activity per cell by decreasing the expression of the AR protein [13]. These results demonstrate that CAG and GGC repeat polymorphisms are contribute to the expression and activity of AR; consequently, these polymorphisms have been studied as etiologic factors in various disorders including polycystic ovary syndrome, breast cancer and benign endometrial cancer in women [19–21].

Saarela et al. performed an association study on the CAG repeat of the *AR* gene and the development of preeclampsia. Their results showed that the *AR* gene CAG repeat length is not a risk factor of preeclampsia, but may be the association between the shortest CAG repeats and preeclampsia [22]. However, influences of GGC repeats and the c.211G>A in the *AR* gene have not yet been reported in preeclampsia. Moreover, influence of CAG repeats in the *AR* gene also has not been confirmed in other ethnic groups.

In this study, we confirmed that CAG repeat in the maternal *AR* gene had no association with the development of preeclampsia in Korean women. This result was agreed with that reported by the prior study [22]. Prior study also reported that children born after preeclamptic pregnancy showed shorter CAG repeat lengths than children born after normal pregnancy and the shortest CAG repeat lengths were found only in the preeclamptic women [22]. However, we could not investigate this polymorphism in participants' children because subjects of this study included only pregnant women. Moreover, the shortest CAG repeat lengths were detected in both of the two groups in this study. Therefore, further genetic and epidemiological studies are required to clarify this discrepancy about the involvement of CAG repeats in preeclampsia.

The important findings of our study were observed in the GGC repeats and c.211G>A. Longer GGC repeats and variant allele of c.211G>A in the maternal *AR* gene showed a greater tendency in preeclamptic women than in normotensive women. These results indicate that the GGC repeats and c.211G>A may be one of the genetic factors that significantly contribute to the risk of preeclampsia. Moreover, the highest risk of preeclampsia was found among women with the particular haplotype as CAG>20/GA/GGC>16 and pregnancies with haplotype such as CAG>20/AA/GGC>16 detected only in the preeclamptic women. Therefore, we suggest that the haplotypes in the *AR* gene such as CAG>20/GA/GGC>16 and CAG>20/AA/GGC>16 could be a suitable biomarker for identifying pregnant women genetically susceptible to preeclampsia in Korean women. However, this study is limited by relatively small sample size, inclusion of only Korean patients, and absent of data correlating *AR* polymorphisms and variations in AR protein levels. Therefore, further studies are needed to explore the exact mechanisms by which these polymorphisms influence susceptibility to preeclampsia in larger and more diverse ethnic populations.

Conclusions

To the best of our knowledge, this is the first study showing the association of the *AR* gene polymorphisms with an

increased risk of preeclampsia in Korean women. In the current study, we found that longer GGC repeats and c.211G>A variant in the *AR* gene were significantly associated with the risk of preeclampsia. However, a study group including larger and more diverse ethnic populations is needed to confirm the exact mechanisms by which these polymorphisms influence susceptibility to preeclampsia.

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