Angiotensinogen G(–6)A Polymorphism Is Associated With the Elevation of Blood Pressure in the Hypertensive Disorders of Pregnancy

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The objective of the present study was to determine whether angiotensinogen G(–6)A polymorphism is associated with the elevation of blood pressure (BP) in the hypertensive disorders of pregnancy in Korean population. The subjects included 201 cases with the hypertensive disorders of pregnancy and 160 healthy controls. The medical records of subjects were reviewed. Cases were classified into the four subtypes (transient hypertension, preeclampsia, chronic hypertension, and preeclampsia superimposed on chronic hypertension) by the diagnostic criteria suggested by the National High Blood Pressure Education Program Working Group. Cases were also divided into the high and low BP group by the elevation of BP (diastolic BP greater than or equal to 110 mmHg). Maternal angiotensinogen G(–6)A polymorphism was determined by restriction fragment length polymorphism. Frequencies of AA genotype were significantly higher in the high than in the low BP group in the preeclampsia, superimposed preeclampsia, and the combined group (N = 201), suggesting that the angiotensinogen G(–6)A allele was significantly associated with the elevation of BP in the hypertensive disorders of pregnancy among South Korean women. The present findings imply that the elevation of BP can serve as an endophenotype for a spectrum of hypertensive conditions in pregnancy.

The hypertensive disorders of pregnancy are characterized by high blood pressure (BP) complicating pregnancy and occur in about 5 to 22% of pregnancies (ACOG practice bulletin, 2002; ACOG technical bulletin, 1996; National High Blood Pressure Education Program Working Group, 2000). The hypertensive disorders of pregnancy are a major cause of maternal and fetal morbidity and mortality (National High Blood Pressure Education Program Working Group, 2000).

Recent population-based family studies have documented that genetics significantly contribute to the development of the hypertensive disorders of pregnancy (Cnattingius et al., 2004; Skjærven et al., 2005). Consistent with these large family studies, molecular studies have reported genes associated with hypertension in pregnancy (Bashford et al., 2001; Guo et al., 1997; Ward et al., 1993). Among the candidate genes, Angiotensinogen gene (AGT) on chromosome 1q42–43 has been most widely studied because AGT was considered to affect sodium and water homeostasis and the regulation of body-fluid volume (Morgan et al., 1996).

Although it is plausible that AGT variants are involved in the pathogenesis of the hypertensive disorders of pregnancy, linkage and association studies conducted so far have produced mixed results. As one of the early association studies of AGT, Ward et al. (1993) found that the AGT T235 allele is significantly associated with preeclampsia and plasma concentration of angiotensinogen in Caucasian and Japanese samples. As the AGT T235 allele is known to be associated with chronic hypertension also (Jeunemaitre et al., 1992), Ward et al.'s study (1993) suggests that AGT T235 may exert pleiotropic effects on development of preeclampsia and chronic hypertension.

Since the Ward et al. study (1993), some studies have yielded positive associations (e.g., Caulfield et al., 1996; Jeunemaitre et al., 1997), while others, negative associations (e.g., Bashford et al., 2001; Guo et al., 1997; Morgan, Crawshaw, et al., 1999; Suzuki et al., 1999).

We argue that the inconsistency in the results of association studies of AGT variants may be in part attributable to the etiological and nosological heterogeneity in the phenotype of the hypertensive disorders

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of pregnancy. To search susceptibility genes of complex diseases like the hypertensive disorders in pregnancy, the use of an endophenotype strategy to identify subclinical traits might yield consistent results because endophenotypes are more directly influenced by gene effects and tend to be simpler than the disease phenotype itself.

In the present study, using an endophenotype strategy, we attempted to identify AGT polymorphisms involved in a variety of clinical conditions that are currently classified in different categories of hypertension in pregnancy. Given that the elevation of BP is the one symptom that is common to a broad spectrum of the hypertensive disorders in pregnancy, we hypothesized that the elevation of BP could be an intervening phenotype for the hypertensive disorders of pregnancy. In the present article, we examined whether AGT G(–6)A was associated with the elevation of BP across the subtypes of the hypertensive disorders of pregnancy as well as in the combined group of all subtypes. The G(–6)A polymorphism is located in the core promoter of AGT with two other polymorphisms, C(–532)T and A(–217)G, and has been reported to affect the basal transcription rate of the gene (Inoue et al., 1997). Recent studies have shown that AGT M235T is in nearly complete linkage disequilibrium with AGT G(–6)A in Caucasians and Japanese (Inoue et al., 1997; Jeunemaitre et al., 1997), suggesting that all associations reported for T235 can be directly extended to A(–6).

Materials and Methods

Subjects

Subjects included Korean pregnant women who were admitted to the labor and delivery unit of the Seoul National University Hospital, Seoul, Korea between May 1998 and March 2002. We reviewed the medical records of all subjects and identified cases with hypertensive disorders. We selected cases who were delivered of live-born neonates with more than 24 weeks of gestation. When a woman had experienced two or more pregnancies with hypertensive disorders, the earliest pregnancy was included in data analyses. This procedure yielded 201 cases. Control subjects included 160 normotensive and healthy pregnant women who had no history of medical or surgical complications. All of the control subjects were delivered of the full-term singletons.

Informed consents were obtained from all subjects, and the institutional review board of the Seoul National University Hospital approved the present study.

Diagnostic Classifications

To classify patients with the hypertensive disorders of pregnancy, we adopted the new classification system released from the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy (2000). The new classification system suggested that the hypertensive disorders of pregnancy include preeclampsia, chronic hypertension, transient hypertension, and preeclampsia superimposed on chronic hypertension (superimposed preeclampsia). Briefly, preeclampsia was diagnosed when the gestational BP reached 140/90 mmHg or higher and proteinuria (greater than or equal to 1+ by dipstick or greater than or equal to 300 mg/24 hour urine) occurred after the 20th week of gestation. Transient hypertension was diagnosed when BP had been elevated to 140/90 mmHg or higher without proteinuria and returned to normal by 12 weeks postpartum. Chronic hypertension was diagnosed when hypertension was documented before pregnancy or before the 20th week of gestation and the elevated BP persisted after 12 weeks post partum. Superimposed preeclampsia was diagnosed when hypertension had been documented before pregnancy or before the 20th week of gestation and preeclampsia was developed during pregnancy in addition to the preexisting hypertensive condition.

All cases and controls were assigned to the high and low BP group. Subjects were allocated to the high BP group when their diastolic BP had ever been recorded as 110 mmHg or higher during pregnancy and when they were given hydralazine intravenously for the elevation of BP. Subjects were assigned to the low BP group when their diastolic BP had been maintained lower than 110 mmHg throughout pregnancy. The diastolic BP cutoff of 110 mmHg was chosen as it is used as a diagnostic criterion of BP to classify severe preeclampsia (the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy, 2000; ACOG practice bulletin, 2002) and because administration of hydralazine is necessary when the BP reaches 110 mmHg. For the purpose of the present study, the senior author (J. K. J.) who was blind to the genotype data examined the medical records of all subjects to determine whether diagnostic criteria were reliably applied to the subjects and whether the subjects were allocated accurately in terms of BP.

Genotyping

Maternal blood was drawn at the time of admission and the blood was centrifuged and stored at –70°C until DNA extraction. Maternal genomic DNA was extracted by the Puregene™ System (Gentra Systems, Inc., Research Triangle Park, NC) from the cellular pellet after centrifugation of the whole blood. For genotyping, maternal genomic DNA was amplified using primers 5'–AATAGGGCATCGTGACCC–3' and 5'–ACCTTCTGCTGTAGTACC–3' (Hegele et al., 1998). The digestion of the PCR product with *Mva I* yielded unfragmented 65 base-pair (G allele) or fragmented 43 and 22 base-pair segments (A allele). Genotypes were determined by the electrophoresis on 3% agarose gel containing ethidium bromide.

Statistical Analysis

We performed one-way ANOVA with Tukey's post hoc comparison to analyze continuous variables and

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Table 1

Maternal Age and Nulliparity by Diagnostic Group

	Transient hypertension	Preeclampsia	Chronic hypertension	Superimposed preeclampsia	Control
N	37	118	18	28	160
Maternal age (yr)					
Mean	30.5	30.5	32.1	35.1*	30.8
SD	± 3.4	± 4.0	± 3.9	± 5.3	± 4.1
Nulliparity					
N	23	90†	6	8†	82
%	62	76	33	29	51

Note: * $p < .05$, significantly different from other groups by Tukey's post hoc comparison. † $p < .05$, significantly different from control group by chi-square test.

chi-square test or Fisher's exact test to analyze categorical variables. Logistic regression analyses were conducted to correct the confounding factors for the analysis of proportion. We used SPSS for Windows (Release 11.0.1, SPSS Inc.) programs for computation. A p value of less than .05 was considered to be statistically significant.

Results

Age and Nulliparity of Subjects

Table 1 presents maternal age and the proportion of nulliparity of subjects by diagnostic group. Tukey's post hoc comparisons revealed that mothers in the superimposed preeclampsia group were significantly older than those in other groups. The preeclampsia group showed a higher proportion of nulliparity than the control. These results were consistent with the literature of preeclampsia showing an increased risk for development of hypertension among nulliparous women (Duckitt & Harrington, 2005).

Association With Angiotensinogen G(–6)A

Table 2 shows the distributions of genotype and allele frequencies of AGT G(–6)A polymorphism by diagnostic group and BP group. Previous studies have reported that the frequency of AGT A(–6) allele was much higher in Mongoloids than in Caucasians (Japanese = 74%, Canadian Oji-Cree = 85%, Caucasians = 38–54%; Hegele et al., 1998; Jeunemaitre et al., 1997). The higher frequency of A allele as compared to that of G allele in Table 2 well reflected the frequency distribution of A allele in the Korean population. For all groups, the distributions of genotype did not significantly differ from Hardy–Weinberg equilibrium.

Table 3 presents genotypic frequencies by diagnostic group and BP group. For all cases (N = 201), the frequency of AA genotype was significantly greater in the high than in the low BP group. When we divided cases into the four subtypes, the difference in the frequency of AA genotype between the high and the low BP group attained a statistical significance in preeclampsia and superimposed preeclampsia. For the transient and chronic hypertension group, however, odd ratios were not calculable for the absence of cases in the high BP group.

Discussion

Recently, the use of endophenotypes has emerged as an important strategy in the study of complex genetic diseases. The significant associations between the

Table 2

Genotype and Allele Frequencies of Angiotensinogen G(–6)A by Diagnostic Group and BP Group

	Transient hypertension		Preeclampsia		Chronic hypertension		Superimposed preeclampsia		Control
Genotype	Low (n = 37)	High (n = 0)	Low (n = 73)	High (n = 45)	Low (n =16)	High (n = 2)	Low (n = 7)	High (n = 21)	(n = 160)
AA	23	—	44	36	11	2	2	16	104
	(62%)	—	(60%)	(80%)	(69%)	(100%)	(29%)	(76%)	(65%)
AG	14	—	27	8	3	0	4	3	51
	(38%)	—	(37%)	(18%)	(19%)	(0%)	(57%)	(14%)	(32%)
GG	0	—	2	1	2	0	1	2	5
	(0%)	—	(3%)	(2%)	(13%)	(0%)	(14%)	(10%)	(3%)
A allele	81%	—	79%	89%	78%	100%	57%	83%	81%
G allele	19%	—	21%	11%	22%	0%	43%	17%	19%

Note: Low = low BP group; high = high BP group. See the text for the definition of the high and low BP group.

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Table 3

Genotypic Distribution by Diagnostic Group and BP Group

Diagnosis	*N*	High BP		Low BP		*p*†	Adjusted *p* value‡	Adjusted OR‡	Adjusted 95% CI‡
		AA	AG + GG	AA	AG + GG				
Transient	37	0	0	23	14	—	—	—	—
Chronic	18	2	0	11	5	> .95	> .95	—	—
Preeclampsia	118	36	9	44	29	< .05	< .05	2.53	1.05–6.07
Superimposed	28	16	5	2	5	.06	< .05	14.65	1.33–161.15
All cases	201	54	14	80	53	< .01	< .01	2.61	1.30–5.22
Controls	160	0	0	104	56	—	—	—	—
All subjects	361	54	14	184	109	< .05	< .05	2.30	1.22–4.35

Note: Transient = transient hypertension; chronic = chronic hypertension; superimposed = superimposed preeclamsia. OR = odds ratio; CI = confidence interval. See the text for the definition of the high and low BP group. †By Fisher's exact test; ‡adjusted for maternal age and nulliparity by logistic regression analysis.

AGT A(–6) allele and the elevation of BP across the subtypes of hypertension in pregnancy found in the present study suggest that the AGT A(–6) allele may be involved in a broad spectrum of the hypertensive disorders during pregnancy through an intermediate phenotype, BP. The question of how different genes act in the pathways between BP and each of the subtypes of the hypertensive disorders in pregnancy still remains to be resolved, however.

Given that the AGT A(–6) allele has been shown to be associated with BP and chronic hypertension (Brand-Herrmann et al., 2004; Iwai et al., 1995), the present finding indicates that the role of the AGT A(–6) allele in the etiology of chronic hypertension may be extended to the pregnancy-induced hypertension. Furthermore, recent studies have reported that hypertension in pregnancy is associated with increased risk of later development of ischaemic heart disease (Wikstrom et al., 2005), and hypertension and stroke (Wilson et al., 2003). It may be that the AGT A(–6) allele is responsible for a general susceptibility to hypertension as a whole.

The finding of an endophenotype for the hypertensive conditions in pregnancy is an important step in understanding the genetic architecture of the hypertensive disorders in pregnancy as it allows the genetic dissection of the disorders and assists to establish an etiology-based diagnosis and classification of the disorders.

It has been proposed that the AGT T235 allele predisposes women toward impaired pregnancy-induced physiological change, leading to development of preeclampsia (Jeunemaitre et al., 1992; Ward et al., 1993). Jeunemaitre et al. (1992) postulated that increased concentration of plasma angiotensinogen in individuals carrying AGT T235 variant could result in increased production of angiotensin II, which in turn could lead to promoting vascular hypertrophy. Ward et al. (1993) further suggested that this vascular hypertrophy, by hampering the expansion in plasma volume that occurs in normal pregnancies, could cause circulatory maladaptation among pregnant women with hypertension carrying AGT T235 variant. Morgan, Craven, et al. (1999) compared spiral artery morphological changes in first-trimester decidual samples between women carrying AGT T235 and those carrying AGT M235. In support of the role of the AGT T235 allele in the physiological change that occurs early in pregnancy, Morgan, Craven, et al. (1999) found an impaired physiological remodeling in women carrying AGT T235 as compared to those carrying AGT M235.

As Lalouel and Rohrwasser (2002) maintained, the main pitfall of an association study is the possibility of false-positive results arising from population admixture and the small sample size. As Koreans are an ethnically very homogeneous population, the issue of population stratification is not a concern in the present study. However, the small number of cases especially in the transient and chronic hypertension group points a need to increase the sample size to substantiate our findings. A population-based case-control design would be useful to overcome the limitations of the present study.

As noted earlier, there are substantial ethnic variations in allele frequencies of AGT G(–6)A. It would be interesting if the relationship between the AGT A(–6) allele and the elevation of BP in a variety of hypertensive conditions in pregnancy found in the present study can be replicated in other racial groups.

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