Original Article

The M235T Variant of the Angiotensinogen Gene Is Related to Development of Self-Reported Hypertension during Pregnancy: The Prospect-EPIC Cohort Study

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Angiotensinogen gene (*AGT*) M235T polymorphism is associated with an increased risk of hypertension. It is unknown whether this mutation also leads to an increased risk of development of high blood pressure (BP) in pregnancy. The aim of this study was to investigate the association of this polymorphism with elevated blood pressure during pregnancy in a population of healthy Dutch women. We studied a randomly selected sample of 1,736 middle-aged women who participated in a prospective cohort study of 17,357 Dutch women. After excluding those who had never been pregnant or those with missing data, 429 women with and 921 women without a history of elevated BP during pregnancy remained for further analyses. History of hypertension in pregnancy was assessed using a questionnaire, and confirmed cases varied in severity from mild blood pressure elevation to pre-eclampsia. Individuals with the TT genotype were more likely to have had a history of elevated BP during pregnancy than those with the MM genotype (odds ratio [OR]=1.43; 95% confidence interval [CI], 1.02–2.01; *p*=0.04). In heterozygote individuals (MT) an increased risk was found, which did not reach statistical significance (OR=1.24; 95% CI, 0.96–1.60; *p*=0.11). Under both dominant and additive genetic models, the M235T polymorphism was associated with a history of elevated blood pressure during pregnancy, with ORs of 1.29 (95% CI, 1.01–1.64; *p*=0.04) and 1.20 (95% CI, 1.02–1.42; *p*=0.03), respectively. The findings of this study among Caucasian Dutch women, aged 49 to 70 years, demonstrated that the presence of the T allele of the M235T polymorphism in the *AGT* is associated with self-reported hypertensive disorders in pregnancy. (*Hypertens Res* 2008; 31: 1299–1305)

***Key Words*: polymorphism, angiotensinogen gene, risk factors, hypertension, pregnancy**

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Introduction

Angiotensinogen (AGT) is a liver protein that interacts with renin to produce angiotensin I, the precursor of angiotensin II. Angiotensinogen levels directly relate to arterial blood pressure (BP) and have been shown to be modified by variants in the angiotensinogen gene (*1*). The molecular variant (M235T) of the AGT gene (*AGT*), encoding the presence of threonine instead of methionine at residue 235 of the mature protein, has been associated with higher plasma AGT levels and higher BP in patients homozygous for the T allele (*1–4*). In a meta-analysis, the TT genotype in comparison with the MM reference group was associated with a 32% increase in risk of hypertension in white but not in non-white people (*5*). As this polymorphism has been postulated to play an important role in the pathogenesis of hypertension, it is plausible to consider it as a candidate as well for susceptibility to the severe hypertensive disorders of pregnancy, which affect up to 8% of all pregnancies (*6*, *7*).

Hypertensive disorders of pregnancy constitute a major cause of maternal and perinatal morbidity and mortality worldwide (*6*). Hypertension in pregnancy can either be chronic hypertension, or a sign of a disorder secondary and specific to pregnancy, called gestational hypertension, or, when proteinuria coincides with it, preeclampsia. The diagnosis of both gestational hypertension and preeclampsia is based on sustained hypertension in (the second half of) pregnancy in a formerly normotensive woman. The etiology of gestational hypertension and preeclampsia is not precisely known. However, it is acknowledged that these are not monocausal disorders but the result of a complex interaction between maternal constitutional factors, fetal factors, placental factors and pregnancy-specific changes (*8*, *9*). Various association studies in various ethnic populations have been performed to reveal (mostly maternal) genetic factors contributing to gestational hypertension and preeclampsia. The angiotensinogen gene is considered to be one of the candidate maternal "susceptibility" genes for gestational hypertension and preeclampsia. Although a few studies have found an association between the *AGT* M235T polymorphism and pregnancy-related hypertension in Caucasians (*10*, *11*) and Japanese (*12*), conflicting results have also been reported in Caucasians (*13*), East Asians (*14*), Black Americans (*15*), and Hispanics (*16*). By using a large population–based study of middle-aged Dutch women, we set out to examine the relationship between the M235T polymorphism of the angiotensinogen gene and pregnancy-related hypertension.

Methods

Population

The study population consisted of participants in the Prospect-EPIC cohort, which is one of the two Dutch contributions of the European Prospective Investigation into Cancer and Nutrition (EPIC). Participants were recruited between 1993 and 1997 among women living in Utrecht and the vicinity who attended the regional population–based breast cancer–screening program. A total of 17,357 women, aged 49–70 years, were included. At enrolment all women underwent a physical examination and completed a general questionnaire relating to lifestyle and medical factors, including pregnancy complications. All women signed an informed consent form prior to study inclusion. The study was approved by the Institutional Review Board of the University Medical Center Utrecht.

Design

From the 17,357 women in the total cohort we randomly selected a 10% sample for laboratory and DNA analyses, as explained elsewhere in detail (*17*). Of the 1,736 women, the ones with missing questionnaires or blood or DNA samples ($n=77$) were excluded from the analyses. Women who had never been pregnant were also excluded ($n=160$). Those who reported an onset of hypertension before the age of 45 years were excluded from the analyses ($n=149$), because no distinction could be made between development of elevated BP during pregnancy and presence of elevated BP before pregnancy. This resulted in 1,350 women, 429 with and 921 without a history of elevated BP during pregnancy.

The general questionnaire contained questions on demographic characteristics, lifestyle habits, obstetric and gynecological history and past and current morbidity. One of these questions was: "did you suffer from elevated BP during pregnancy?" Those women who answered in the affirmative to this question were considered to have had elevated BP during pregnancy. Since no information was obtained on the actual BP levels, an affirmative response to the question likely led to inclusion of women with BP elevations in pregnancy ranging from mild to severe (pre-eclampsia). Upon enrolment in the study, systolic BP (SBP) and diastolic BP (DBP) were measured in duplicate, and the mean value was calculated. Furthermore, height and weight were measured without shoes and wearing light indoor clothing to compute the body mass index (BMI), which was defined as weight divided by height squared (kg/m^2). Presence of hypertension was defined as a measured SBP $\geq$140 mmHg and/or a DBP $\geq$90 mmHg and/or a self-reported physician diagnosis.

Genetic Analysis

Genetic analysis was performed at the Cardiovascular Genotyping (CAGT) laboratory of the Department of Internal Medicine of the University Hospital Maastricht. Genomic DNA was extracted from buffy coats with the use of the QIAamp® Blood Kit (Qiagen Inc., Valencia, USA). Genotyping of the polymorphisms was performed using a multilocus genotyping assay for candidate markers of cardiovascular dis-

Table 1. General Characteristics of Cases with a History of Elevated Blood Pressure in Pregnancy and Controls

	History of elevated blood pressure in pregnancy (n=1,350)		p-value
	Cases	Controls	
Total (n (%))	429 (31.8%)	921 (68.2%)	—
Age (years)[#]	56.9±6.1	57.2±6.1	0.51
Body mass index (kg/m^2)[#]	26.4±4.1	25.6±3.9	<0.01
Weight (kg)[#]	71.8±11.8	68.9±10.8	<0.01
Height (cm)[#]	164.8±5.9	164.0±5.9	0.03
Waist to hip ratio[#]	0.793±0.058	0.789±0.056	0.17
Hypertension (%)*	59.2	34.0	<0.01
Systolic blood pressure (mmHg)	137.4±19.3	130.4±20.1	<0.01
Diastolic blood pressure (mmHg)	81.6±10.3	77.8±10.7	<0.01
Presence of diabetes (%)	2.6	2.0	0.55
Presence of hypercholesterolemia (%)	4.4	3.9	0.66
Smoking status (%)			
Past	36.8	35.0	0.50
Current	21.0	23.5	0.33
Pack-years[§]	6.3±9.0	6.9±9.7	0.34
Current alcohol consumption (%)	89.3	87.8	0.50
Menopausal status (%)	73.9	72.7	0.54
Age at menopause transition (years)[#]	47.5±5.9	47.3±5.7	0.74
Total cholesterol (mmol/L)[#]	5.93±0.99	5.85±0.98	0.15
HDL cholesterol (mmol/L)[#]	1.57±0.38	1.59±0.41	0.41
LDL cholesterol (mmol/L)[#]	3.96±0.92	3.91±0.94	0.38
Glucose (mmol/L)[#]	4.6±1.4	4.5±1.4	0.27
Genotype frequency			
T235T	83	147	
M235T	214	439	
M235M	132	335	
Allele frequency			
T	380	733	
M	478	1,109	

HDL, high-density lipoprotein; LDL, low-density lipoprotein. [#]Mean±SD. *Defined as a systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and/or questionnaire positive. [§]The number of packs of cigarettes smoked per day by the number of years the person has smoked.

ease risk (Roche Molecular Systems Inc., Pleasanton, USA) (*18*). Investigators involved in genotyping were blinded to the case-control status of the participants. A random double-check was performed to detect potential genotyping errors and was 100% concordant with the original genotype.

Data Analysis

Data analysis was conducted using SPSS-12.0.1. Sample characteristics were examined according to case status. Means and standard deviations were computed for normally distributed variables. We used Student's t-test to test for significant differences in continuous variables between cases and controls, while we tested the significance of differences in proportions by applying the χ^2 statistic. Hardy-Weinberg equilibrium was tested with the χ^2 test in the control group. Multivariate logistic regression models were used to study the association of the *AGT* M235T polymorphism with a history of elevated BP during pregnancy under different genetic models. Allele frequencies were estimated by gene counting. A value of $p<0.05$ (2-tailed) was considered significant.

Since the information on the history of high BP in pregnancy was collected at the baseline examination of the Prospect-EPIC study (long after the last pregnancy), those with chronic hypertension may have recalled high BP in pregnancy better than those women who did not suffer from hypertension at baseline (*i.e.*, there may have been a recall bias). To examine this, we repeated the analyses after subjects with hypertension at baseline were excluded (n=255).

Table 2. Relation of the *AGT* M235T Genotypes and History of Elevated Blood Pressure in Pregnancy

Genotypes	Crude: model 1			Adjusted: model 2*		
	Odds ratio	95% CI	*p*-value	Odds ratio	95% CI	*p*-value
M235M	1.00			1.00		
M235T	1.24	0.96–1.60	0.11	1.26	0.96–1.66	0.09
T235T	1.43	1.02–2.01	0.04	1.55	1.09–2.20	0.01

AGT, angiotensinogen gene; CI, confidence interval. *Adjusted for body mass index and presence of hypertension.

Table 3. Association of the *AGT* M235T Polymorphism and History of Elevated Blood Pressure in Pregnancy under Different Genetic Models

Mode of inheritance	Crude: model 1			Adjusted: model 2*		
	Odds ratio	95% CI	*p*-value	Odds ratio	95% CI	*p*-value
Dominant (T-carriers *vs*. MM)	1.29	1.01–1.64	0.04	1.33	1.03–1.72	0.03
Recessive (TT *vs*. M-carriers)	1.26	0.94–1.70	0.12	1.35	0.99–1.84	0.06
Additive (T allele *vs*. M allele)	1.20	1.02–1.42	0.03	1.25	1.05–1.48	0.01

AGT, angiotensinogen gene; CI, confidence interval. *Adjusted for body mass index and presence of hypertension.

Results

The genotype distribution among control subjects was in Hardy-Weinberg equilibrium (χ^2=0.025; p=0.87). Characteristics of participants and genotype and allele frequencies in cases and controls are shown in Table 1. Within the control group, the T allele frequency was 40% and the genotype distributions were 16%, 47.7%, and 36.3% for the TT, MT, and MM genotypes, respectively. In our study population, the prevalence of a history of high BP in pregnancy was 31.8% (n=429). The high prevalence was most likely due to the inclusion of not only women with genuine preeclampsia but also women with more common nonproteinuric gestational hypertension, or women with brief and modest elevations of BP during pregnancy.

Compared to those without, women with a history of elevated BP during pregnancy had a higher BMI, weight, height, SBP, and DBP, and presence of chronic hypertension was more common. Among the control subjects, only BMI and the presence of chronic hypertension were significantly related to the polymorphism. Under the dominant model, among the variant carriers, 51.2% (n=300) had low BMI (≤25 kg/m^2) and 48.8% (n=286) had high BMI (>25 kg/m^2), but among the MM carriers 44.5% (n=149) had low BMI and 55.5% (n=186) had high BMI, and the association was significant (p=0.050). Chronic hypertension was related to the *AGT* M235T polymorphism under the additive (p=0.016) and pairwise (TT/MM; p=0.053) genetic models. Although these factors could be considered as potential confounding factors in our data analyses, one may also argue that these factors are intermediate phenotypes in the causal pathway. In addition, because genes are randomly assigned at conception (Mendelian randomization), confounding by lifestyle-related factors (or intermediate phenotypes) should not be a problem in genetic association studies (*19*). Therefore we showed crude and adjusted results (Tables 2 and 3).

Individuals with the TT genotype were more likely to have had a history of elevated BP during pregnancy than those with the MM genotype (odds ratio [OR]=1.43; 95% confidence interval [CI], 1.02–2.01), but for heterozygote individuals the difference did not reach the level of statistical significance. After adjustment for BMI and presence of hypertension, those with the TT genotype were 55% more likely to have had a history of elevated BP during their previous pregnancies (OR=1.55; 95% CI, 1.09–2.20; Table 2).

The crude ORs of risk of elevated BP in pregnancy were 1.29 (95% CI, 1.01–1.64) for the dominant genetic model and 1.20 (95% CI, 1.02–1.42) for the additive model. Adjusting for BMI and hypertension increased the ORs to 1.33 (95% CI, 1.03–1.72) and to 1.25 (95% CI, 1.05–1.48) for the dominant and the additive genetic models, respectively. Under a recessive model we did not find a statistically significant relationship in the crude estimate (OR=1.26; 95% CI, 0.94–1.70). After adjustment, only a borderline effect was seen in model 2 (Table 3).

We repeated the analysis of the relation between the polymorphism and history of high BP in pregnancy using different genetic models, after exclusion of subjects with hypertension at baseline (n=783) to control for potential recall bias. In doing so, the magnitude of the effect estimates increased under different genetic models. The BMI-adjusted OR for the TT genotype *vs*. the MM genotype increased to 1.77 (1.20–2.61). Likewise, adjusted ORs using the dominant, recessive and additive models rose to 1.42 (1.05–1.90), 1.51 (1.08–2.13), and 1.33 (1.10–1.61), respectively.

Discussion

In this population-based study among women aged 49 to 70 years, we found that individuals with the TT genotype were more likely to have had a history of elevated BP during pregnancy than those with the MM genotype. Furthermore, under dominant and additive inheritance models, the *AGT* M235T polymorphism was associated with increased risk of BP elevation in pregnancy.

Some limitations of our study need to be addressed. The information on history of high blood pressure during pregnancy was obtained by questionnaire when the participants were at or above middle age. This may have led to misclassification of the outcome. The question was not designed to gather only information on the most severe hypertensive disorder of pregnancy, *i.e.*, preeclampsia. Therefore, milder variants of BP elevation in pregnancy were also included. One might question the effect of including these milder variants on the validity and magnitude of our findings. If only the more severe hypertensive disorder of pregnancy, *i.e.*, preeclampsia, is related to the T235 allele of the *AGT*, then the magnitude of our findings is clearly an underestimation of the truth. However, the direction of the relation would still be valid in such a case. Unfortunately, BP levels during pregnancy were not available. Despite the impossibility of precisely classifying hypertension in pregnancy on the basis of this information, the M235T polymorphism was related to a positive history of high BP in pregnancy. We therefore assume that the true relationship may actually be stronger than the one we observed, rather than attenuated. Another consideration is that recall bias may have affected the association. However, our stratified analysis showed that the magnitude of the relation among normotensive women was higher than that of the entire group, and therefore, the reported relation does not seem to be biased. The strengths of the study were its population-based nature and large sample size, which allowed us to evaluate the association using different inheritance models. Moreover, we avoided misclassification of exposure (genotypes) by using standard laboratory protocols and performing a random double-check to detect potential genotyping errors, and we confirmed that the *AGT* M235T genotypes were in the Hardy-Weinberg equilibrium. Furthermore, the distribution of *AGT* M235T genotypes in our study was similar to that found in other studies among Dutch middle-aged subjects (*20*, *21*)

In the first report on this association, 45% of the controls and 77–80% of the preeclampsia cases were homozygous for the mutation M235T polymorphism (*10*). This result was replicated in subsequent case-control studies in Japanese (*12*) and Caucasian women (*11*). Our results expand the evidence for the association to a larger study and an additional Caucasian population. However, there are also studies which have failed to confirm the association (*13–16*, *22*). These discrepancies could be explained by differences in disease severity (*i.e.*, the proportions of women with preeclampsia *vs*. gestational hypertension) among the various study populations, as well as by the different ethnic backgrounds of subjects, if different disease–causing alleles predominate in different study populations, or if there exists a variation in the degree of linkage disequilibrium between marker and disease alleles (*23*). In addition, inadequate sample sizes and lack of statistical power could be another reason for the inconsistent results in some studies (*13*, *16*, *22*). Modification of the association by other environmental factors in different populations, population stratification, different definitions used for the outcome (*10*, *12*) and genotyping errors (*24*) could also explain, in part, the failure to replicate the positive findings.

Hypertension during pregnancy, even in its most mild form, is a major pregnancy complication, causing premature delivery, fetal growth retardation, abruption placenta, and fetal death, as well as maternal morbidity and mortality (*6*, *25*). These risks are higher in preeclampsia, the most severe form of BP elevation in pregnancy. It has been shown that the likelihood of progression from a mild gestational hypertension to preeclampsia, which is a leading cause of maternal and fetal mortality, varies from 15% to 26% (*25*). Gestational hypertension has been recognized for centuries; however, the etiology of this syndrome remains uncertain. Early in pregnancy the spiral arteries are transformed from thick-walled, muscular vessels to saclike flaccid vessels. This transformation involves invasion of the spiral arteries by endovascular trophoblast cells of the placenta, which remains incomplete in women in whom preeclampsia eventually develops, with the vessels remaining thick-walled and muscular (*6*). This remodeling of the abnormal spiral arteries may lead to reduced uteroplacental blood flow, which could initiate the cascade of events leading to preeclampsia. It has been reported that the T allele of the *AGT* may be involved in this abnormal remodeling. Therefore, the systemic renin-angiotensin system may not be as important as the local system in this process (*26*).

During the last decade evidence has accumulated that hypertensive disorders of pregnancy, and preeclampsia in particular, are associated with future hypertension and cardiovascular diseases (CVD). Preeclampsia and CVD share chronic hypertension, increased total cholesterol, decreased insulin sensitivity, and increased BMI as common risk factors (*27–29*). Large epidemiological studies have demonstrated that women who have had preeclampsia are at high (up to 7-fold) risk to develop CVD in later life (*30–32*). Apparently, exposure of the women with this phenotype to the additional metabolic and cardiovascular challenges of pregnancy induces transient clinical disease (*i.e.*, preeclampsia), that subsides after pregnancy but is likely to re-emerge later in life as CVD (*33*, *34*). The *AGT* T235T genotype may represent a genetic "susceptibility" for hypertensive disorders in pregnancy, chronic hypertension and atherosclerosis in later life.

In conclusion, the findings of this study among Caucasian Dutch women, aged 49 to 70 years, are in agreement with those studies which previously found that the presence of the T allele of the M235T polymorphism in the *AGT* is associated

with elevated BP in pregnancy.

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