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Preeclampsia Risk and Angiotensinogen Polymorphisms M235T and –217 in African American and Caucasian women

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Abstract

Introduction—Genetic variants of the angiotensinogen gene have been linked to both hypertension and preeclampsia. The M235T polymorphism is more common in hypertension and preeclampsia in some populations. A polymorphism in the angiotensinogen basal promoter region AGT–217 is more common in African Americans with hypertension. We investigated the frequency of M235T and AGT–217 in Caucasian and African American women with and without preeclampsia.

Methods—This was a nested case control study of primiparous women with singleton pregnancies. Genomic DNA from preeclamptic and control subjects underwent PCR amplification and restriction digestion.

Results—The M235T and AGT –217 polymorphisms were both more common in African American women, however the variants were not more common in preeclampsia.

Conclusion—The frequency of angiotensinogen polymorphisms M235T and AGT –217 is different by race, however these polymorphisms are not associated with an increased risk of preeclampsia.

Keywords

preeclampsia; angiotensinogen; polymorphisms; race/ethnicity

Introduction

The heritability of hypertension has been demonstrated in genetic linkage and association studies involving twins and families [1]. Genetic polymorphisms of the renin angiotensin system have been extensively studied [1]. The angiotensinogen polymorphism M235T is more common in Caucasian men with severe hypertension [2]. Though the M235T polymorphism

is frequent in people of African descent (81-91%), it has not been found to be associated with hypertension [3]. There is a clear heritability to preeclampsia [4-6], and there are reports of an increased risk of preeclampsia in some populations with the M235T polymorphism [7,8]. Impaired trophoblastic invasion of maternal spiral arteries in preeclampsia is also associated with the M235T polymorphism [9]. African Americans with hypertension have an increased frequency of a G-A polymorphism at -217 (AGT -217) in the basal promoter region of the angiotensinogen gene [3,10]. The objective of this study was to investigate the relationship and prevalence of the M235T and AGT -217 polymorphisms of the angiotensinogen gene in preeclampsia in African American and Caucasian women.

Methods

Population description

This was a nested case control study of 783 nulliparous women from the Prenatal Exposure Preeclampsia Prevention prospective cohort trial (PEPP) at Magee Women's Hospital in Pittsburgh. The PEPP study was reviewed and approved by the Institutional Review Board.

There were 293 Caucasian and 272 African American women with uncomplicated pregnancy outcomes. Race and ethnicity were determined by self report. The cases and controls were a mix of spontaneous and induced labor. Subjects with preeclampsia included 30 African American and 192 Caucasian women. Preeclampsia was defined by the research criteria recommended by the National High Blood Pressure Education Program: gestational hypertension, proteinuria, and return of all abnormalities to normal by 12 weeks postpartum. Gestational hypertension was defined as systolic blood pressure persistently ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg diastolic for the first time after 20 weeks of gestation. In this study, we determined blood pressure as the average of the last 5 blood pressures obtained in semi-Fowlers position after hospital admission for delivery but before medications or clinical perturbations that would alter blood pressure. Proteinuria was the excretion of ≥ 300 mg of protein in 24 hours, $\geq 2+$ protein on voided urine sample, $\geq 1+$ protein on catheterized urine specimen, or a protein-creatinine ratio of ≥ 0.3 . Control patients had no known prior medical conditions.

Sample size and power calculations

This was an exploratory study that used previously collected banked specimens in order to assess the association of angiotensinogen polymorphisms M235T and -217 with preeclampsia. Sample size and power calculations were based on a single gene effect model with a recessive mode of inheritance, a two sided alpha of 0.05 and 80% power (Quanto).

M235T—Using a population frequency of 40% in the control population based on the work done by Ward, we could detect a 1.7 fold increase in T allele frequency in all preeclampsia subjects compared to all controls, and in Caucasian cases compared to control subjects. The population frequency used for African American subjects was 81% based on the frequency of the allele in the African American population in the study done by Rotmi. Given our sample size of 18 African American preeclampsia cases a 4.2 fold increased frequency of the T allele in preeclampsia subjects compared to control subjects would be detected with a power of 80% and a two sided alpha of 0.05.

-217—Using a population frequency of 10% based on the Jain study of increased hypertension in African Americans with -217, we could detect a 1.6 fold increase in the frequency of the polymorphism in cases compared to controls in the entire study population. Similarly in Caucasian subjects a 1.4 fold increase in the -217 polymorphism in cases compared to controls

could be detected. The population frequency used for African Americans was 19% based on the study done by Jain.

In African American subjects with 30 available cases, a 3 fold increase in the -217 polymorphism would be detected with a power of 80% and a two sided alpha of 0.05.

Analysis of the Genomic DNA

DNA was extracted from peripheral blood leukocytes by using a standard protocol [3,11]. Genotyping was not successful in some samples and the number for each genotype is indicated with that genotype. Negative controls were included in all PCR genotype analyses.

AGT -217

The genomic DNA from cases and control subjects was amplified using 5'-CTC AGT GCT GTC ACA CAC CTA-3' as the forward primer and 5'-AAG TGA CAC CAC CTC CAG TCT TTA GT-3' as the reverse primer. The amplification product (233 bp) contained nucleotides -314 to -82 of the human AGT gene promoter, including the A/G polymorphic site at -217. These amplified fragments were treated with *AluI* to identify the A/G polymorphic site at -217. The restriction enzyme *AluI* (restriction site AGCT) cuts the amplified sequence if nucleoside A is present at -217 and produces 134- and 99-bp fragments. After restriction analysis, the resulting fragments were separated by electrophoresis in a 2.5% agarose gel and stained with ethidium bromide. The nucleotide sequences of the amplified products were determined by sequence analysis to confirm the results of restriction analysis.

AGT M235T

100 ng of genomic DNA was amplified for 35 cycles in a reaction volume of 25 micro Liter containing 0.2 mmol/L of each dNTP, 10 mmol/L Tris HCl (pH 9.0), 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 0.1% Triton X-100, 1 U Taq DNA polymerase, and 25 pmol each of primers 5'-GAT GCG CAC AAG GTC CTG TC-3' (forward) and 5'-CAG GGT GCT GTC CAC ACT GGA CCC C-3' (reverse).

The 303-bp polymerase chain reaction product was exposed to the restriction enzyme *TthIII I* for 16 hours at 65 degrees Celsius and electrophoresed on a 3% agarose gel with ethidium bromide staining. With this primer pair, the M235 allele is visible as an uncut 303-bp fragment and the T235 allele as a cleaved 279-bp fragment.

Statistical Analysis

SPSS software was used for analysis of clinical variables, data were summarized as mean $\pm$ SD. Allele frequencies were determined by gene counting and tested for fit to the expectations of Hardy Weinberg equilibrium by chi square. Statistical significance was accepted at $p < 0.05$. Quanto1.1 was used for analysis of allele and genotype frequency sample size and power [12].

Results

Clinical features of study participants are presented in table 1; maternal age was different by race for both controls and cases, African American women were younger than Caucasian women. However, other preeclampsia related characteristics were similar for African Americans and Caucasians.

The average systolic and diastolic blood pressures were higher in preeclampsia cases compared to normotensive controls. Similarly, the average birth weight and birth weight centile was lower in preeclampsia cases compared to normotensive controls. For both Caucasian and African

American cases, gestational age at delivery was earlier, however there was no difference by race.

-217 AGT genotype and allele frequency

There were 773 participants 551 controls and 222 cases for the -217 AGT analyses with the racial distribution as presented in table 2. The control-case ratio for AGT -217 was 1.4:1 for Caucasians and 9.4:1 for African Americans.

In the overall analysis of cases and controls without regard to race shown in table 2 there were no differences in the genotype or allele frequency distributions. The allele frequencies were in Hardy Weinberg equilibrium among the controls in the entire cohort and within both racial strata. The A allele, AA and AG genotype were significantly more common in African Americans compared to Caucasians, A allele frequency $\chi^2 = 61.6$ p=0.00. Analysis within race comparing cases and controls revealed no difference in the -217 AGT AA genotype and A allele frequency.

M235T genotype and allele frequency

There were 610 participants for M235T analysis; 440 controls and 170 cases with the racial breakdown as shown in table 3. The control-case ratio was quite different by race; 1.6:1 for Caucasians and 11.2:1 for African Americans. The allele frequencies were in Hardy Weinberg equilibrium among the controls in the entire cohort and within both racial groups.

In the overall study population, the large difference in genotype and allele frequency by race confounded the association between preeclampsia cases and normotensive pregnancy controls as shown in tables 3. The TT genotype and T allele frequencies were more common in African Americans while the MM genotype was uncommon. In Caucasians the MM and MT genotypes and M alleles predominated. In the analysis by race, the chi square for the TT genotype was 146.7, p=0.000, (data not shown). Similarly the frequency of T allele was more common resulting in a chi square of 170.0 p= 0.001, (data not shown). We randomly selected 40 of the 218 African American controls to adjust the case-control ratio by race. In this analysis, the chi square for the entire study population was 0.926 and p=0.336, there was no difference in the frequency of the T allele among the randomly selected African American cases. A within race analysis of African American and Caucasian women demonstrated that there was no difference in genotype or allele frequency between cases and controls.

Discussion

Hypertension and preeclampsia are similarly complex and multifactorial disorders with genetic and environmental elements. Numerous and extensive analysis of polymorphisms of the angiotensinogen gene reveal a relationship to severe hypertension and cardiovascular morbidity and mortality [1].

Proposed pathophysiologic mechanisms for both preeclampsia and hypertension include inflammation, increased oxidative stress, endothelial activation and dysfunction. Angiotensinogen polymorphisms are associated with all of these changes.

Angiotensinogen is expressed in many tissues and a broad range of specific cells, it acts as a substrate for renin, and the conversion of angiotensinogen to angiotensin is the rate limiting step in the renin angiotensin cascade. Human studies have demonstrated an association of increased copies of the AGT gene and increased plasma AGT concentrations with elevated blood pressure [13]. Animal studies using transgenic mouse and rat models which overexpress AGT and renin genes similarly show elevated blood pressure, endothelial dysfunction and renal abnormalities [13,14].

The results of our exploratory study are consistent with some but not all prior studies of M235T and preeclampsia; Ward reported an association in a population of Caucasian women in Utah, and Kobashi reported a positive association in a Japanese population [7,8]. Levesque using a large Caucasian French Canadian population did not find an association with M235T, however reported an association with T174M [15].

The Genetics of Preeclampsia Consortium (GOPEC), was a collaborative study involving 10 universities in the United Kingdom that examined the role played by genes previously reported to confer susceptibility to preeclampsia. Seven candidate genes and 28 single nucleotide polymorphisms including M235T were genotyped in 657 women with preeclampsia as well as their families. The GOPEC researchers reported that none of the genetic variants tested conferred a high risk of the syndrome [16].

The GOPEC study did not include AGT -217, and to our knowledge, there are no other studies of this polymorphism and preeclampsia.

Strengths of our study were strict definitions of preeclampsia with chart abstraction providing extensive information on other potential contributing factors. Our study was limited by the small number of black preeclampsia cases. Sample size and power calculations reveal that while the sample size for Caucasian cases was adequate to detect moderate changes in the allele frequencies between cases and controls, much larger allele frequency increases in African American subjects were required due to the smaller sample size. For the AGT -217 polymorphism a post hoc analysis reveals that 370 African American cases and controls would be required to detect a 2 fold increase in allele frequency in preeclampsia subjects compared to controls. Similarly for the M235T polymorphism 159 African American cases and controls would be required to detect a 2 fold increase in allele frequency in preeclampsia subjects compared to controls.

Genetic association studies with small numbers are at risk for both type I and type II errors, thus large studies are required, though it is possible that smaller studies can be combined in meta-analysis to identify genetic effects. Large studies would also provide the ability to analyze haplotypes (a series of alleles found at linked loci on a single chromosome), gene-gene interactions and gene-environment interactions [17]. In a review article about the search for genetic clues to preeclampsia the authors note “the overwhelming majority of gene studies in preeclampsia have investigated women of white Western European descent. Very few studies have included black women which is both surprising and regrettable in view of the high risk of pre-eclampsia in black women and the high incidence of eclampsia in Africa.” [18]

The use of self reported race has been challenged by some researchers in the biomedical sciences. However, a US based study of the genetics of hypertension using genetic cluster analysis of microsatellite markers produced four major clusters that were 99% in agreement with self reported race/ethnicity and ancient genetic ancestry for white, African American, East Asian and Hispanic participants [19].

In addition to increased morbidity in African Americans, genetic variation along racial, ethnic and ancestral lines clearly plays a role in the response to pharmacotherapeutics. It is hoped that advances in knowledge and technologies will permit identification of those at the highest risk and result in decreasing morbidity and mortality.

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References

1. Dickson ME, Sigmund CD. Genetic basis of hypertension: revisiting angiotensinogen. *Hypertension* 2006;48(1):14–20. [PubMed: 16754793]
2. Borecki IB, et al. Associations of candidate loci angiotensinogen and angiotensin-converting enzyme with severe hypertension: The NHLBI Family Heart Study. *Ann Epidemiol* 1997;7(1):13–21. [PubMed: 9034402]
3. Jain S, et al. Angiotensinogen gene polymorphism at –217 affects basal promoter activity and is associated with hypertension in African-Americans. *J Biol Chem* 2002;277(39):36889–96. [PubMed: 12145290]
4. Arngrimsson R, et al. Genetic and familial predisposition to eclampsia and pre-eclampsia in a defined population. *Br J Obstet Gynaecol* 1990;97(9):762–9. [PubMed: 2242360]
5. Chesley LC. The remote prognostic significance of the level of blood pressure in pregnancy. *Clin Exp Hypertens* 1980;2(5):777–801. [PubMed: 7000471]
6. Skjaerven R, et al. Recurrence of pre-eclampsia across generations: exploring fetal and maternal genetic components in a population based cohort. *Bmj* 2005;331(7521):877. [PubMed: 16169871]
7. Kobashi G, et al. Association of a variant of the angiotensinogen gene with pure type of hypertension in pregnancy in the Japanese: implication of a racial difference and significance of an age factor. *Am J Med Genet* 1999;86(3):232–6. [PubMed: 10482871]
8. Ward K, et al. A molecular variant of angiotensinogen associated with preeclampsia. *Nat Genet* 1993;4(1):59–61. [PubMed: 8513325]
9. Morgan T, et al. Angiotensinogen Thr235 variant is associated with abnormal physiologic change of the uterine spiral arteries in first-trimester decidua. *Am J Obstet Gynecol* 1999;180(1 Pt 1):95–102. [PubMed: 9914585]
10. Rotimi C, et al. Polymorphisms of renin-angiotensin genes among Nigerians, Jamaicans, and African Americans. *Hypertension* 1996;27(3 Pt 2):558–63. [PubMed: 8613203]
11. Russ AP, et al. Rapid detection of the hypertension-associated Met235-->Thr allele of the human angiotensinogen gene. *Hum Mol Genet* 1993;2(5):609–10. [PubMed: 8518804]
12. Gauderman WJ. Sample size requirements for matched case-control studies of gene-environment interaction. *Stat Med* 2002;21(1):35–50. [PubMed: 11782049]
13. Kim HS, et al. Genetic control of blood pressure and the angiotensinogen locus. *Proc Natl Acad Sci U S A* 1995;92(7):2735–9. [PubMed: 7708716]
14. Ganten D, et al. Species specificity of renin kinetics in transgenic rats harboring the human renin and angiotensinogen genes. *Proc Natl Acad Sci U S A* 1992;89(16):7806–10. [PubMed: 1502199]
15. Levesque S, et al. Implication of an AGT haplotype in a multigene association study with pregnancy hypertension. *Hypertension* 2004;43(1):71–8. [PubMed: 14638622]
16. Jorgenson E, et al. Ethnicity and human genetic linkage maps. *Am J Hum Genet* 2005;76(2):276–90. [PubMed: 15627237]
17. Romero R, et al. The design, execution, and interpretation of genetic association studies to decipher complex diseases. *Am J Obstet Gynecol* 2002;187(5):1299–312. [PubMed: 12439524]
18. Chappell S, Morgan L. Searching for genetic clues to the causes of pre-eclampsia. *Clin Sci (Lond)* 2006;110(4):443–58. [PubMed: 16526948]
19. Tang H, et al. Genetic structure, self-identified race/ethnicity, and confounding in case-control association studies. *Am J Hum Genet* 2005;76(2):268–75. [PubMed: 15625622]

Table 1

Clinical Features of Controls and Cases

	Normotensive Controls		Preeclampsia Cases	
	African American N=271	Caucasian N=288	African American N=30	Caucasian N=193
Maternal age (years) *	20.8 ± 3.9	24.0 ± 5.2	21.3 ± 6.1	28.1 ± 5.8
Average systolic blood pressure (mmHg) †	121 ± 11.6	120 ± 11.6	160 ± 18.0	157 ± 16.1
Average diastolic blood pressure (mmHg) †	73 ± 7.9	72 ± 7.3	94 ± 10.6	94 ± 10.0
Gestational age at delivery (weeks) †	39.2 ± 2.7	39.5 ± 1.9	36 ± 3.4	35 ± 3.9
Birthweight (grams) †	3204 ± 595	3393 ± 530	2434 ± 825	2298 ± 926
Birthweight centile (%) †	51 ± 26.9	47.8 ± 26.7	31 ± 28.4	28 ± 23.7

Data are mean ± standard deviation

* = p<0.05, Maternal age is different by race p=0.02,

† = p<0.05 difference between controls and cases

Table 2
Angiotensinogen -217 Genotype and Allele Frequency by Race and Case-Control Status

Genotype frequency	Total subject population		Caucasian only		African American only	
	Normotensive controls (n=551)	Preeclampsia cases (n=222)	Normotensive controls (n=283)	Preeclampsia cases (n=192)	Normotensive controls (n=268)	Preeclampsia cases (n=30)
AA	22 (4.0)	7 (3.2)	2 (0.7)	3 (1.6)	20 (7.5)	4 (13.3)
AG	158 (28.7)	58 (26.9)	64 (22.6)	45 (23.4)	94 (35.0)	13 (43.3)
GG	371 (70.3)	157 (70.7)	217 (76.7)	144 (75.0)	154 (57.5)	13 (43.3)
	$\chi^2 = 0.931$, p=0.63		$\chi^2 = 0.87$ p=0.65		$\chi^2 = 2.63$, p=0.27	
Allele frequency						
A	202 (18.0)	72 (16.0)	68 (12.0)	51 (13.3)	134 (25.0)	21 (35.0)
G	900 (82.0)	372 (84.0)	498 (88.0)	333 (86.7)	402 (75.0)	39 (65.0)
	$\chi^2 = 0.97$, p=0.35		$\chi^2 = 0.34$, p=0.56		$\chi^2 = 2.80$, p=0.09	

A allele frequency is significantly increased in African Americans compared to Caucasians, OR 2.81, 95% CI 2.15-3.65

Table 3
Angiotensinogen M235T Genotype and Allele Frequency by Race and Case-Control Status

Genotype frequency	Total subject population		Caucasian only		African American only	
	Normotensive controls (n=440)	Preeclampsia cases (n=170)	Normotensive controls (n=238)	Preeclampsia cases (n=152)	Normotensive controls (n=202)	Preeclampsia cases (n=18)
MM	88 (20.0)	45 (26.5)	80 (33.6)	45 (29.6)	8 (4.0)	0 (0.0)
MT	188 (42.7)	81 (47.6)	119 (50.0)	77 (50.7)	69 (34.2)	4 (22.2)
TT	164 (37.3)	44 (25.9)	39 (16.4)	30 (19.7)	125 (61.9)	14 (77.8)
	$\chi^2 = 7.69, p=0.021^*$		$\chi^2 = 1.06, p=0.59$		$\chi^2 = 2.08, p=0.35$	
Allele frequency						
T	515 (58.6)	169 (49.7)	197 (41.4)	137 (45.0)	319 (79.0)	32 (90.0)
M	364 (41.4)	171 (50.3)	279 (58.6)	167 (55.0)	85 (21.0)	4 (10.0)
	$\chi^2 = 7.86, p=0.005^*$		$\chi^2 = 1.03, p=0.31$		$\chi^2 = 1.8, p=0.18$	

* Chi square value is confounded by large racial difference in allele frequency. T allele frequency is significantly increased in African Americans compared to Caucasians, OR 5.23 95% CI 4.01-6.91.