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Association of maternal angiotensinogen gene M235T polymorphism with preeclampsia in South India: A tertiary care hospital based case-control study

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ABSTRACT

Purpose: M235T polymorphism (rs699) in angiotensinogen (*AGT*) gene is associated with the risk of developing preeclampsia. This case-control study examines the profile of *AGT* M235T polymorphism in preeclamptic and normotensive pregnancies in a South Indian population.
Methods: A total of 300 women comprising of 150 preeclamptic pregnancies and an equal number of normotensive pregnancies were enrolled in the study. *AGT* M235T polymorphism was determined by PCR-RFLP technique. Differences in the distribution of alleles and genotypes among cases and controls was evaluated for statistical significance by means of contingency table.
Results: The presence of *AGT* 235 T allele was found to be associated with the increased risk of developing preeclampsia [$P = 0.003$, odds ratio = 1.69 (95% CI: 1.20–2.38)]. At the genotype level, the odds ratio for the risk of PE was 2.52 (95% CI: 1.25–5.07) and 1.67 (95% CI: 1.05–2.63) in the dominant and recessive models respectively.
Conclusions: Our results support the conclusion that *AGT* M235T polymorphism is associated with increased risk of developing preeclampsia in the South Indian population. This is the first study to report a positive association between *AGT* M235T polymorphism and preeclampsia in the Indian population.

1. Introduction

Preeclampsia (PE) is a pregnancy related complication involving hypertension and proteinuria. It is a major obstetric issue worldwide. The global prevalence of PE is 5–8%, while that in India is about 28.7% (Roberts and Lain, 2002; Agrawal and Walia, 2014). PE is a major public health issue as it contributes to pregnancy related maternal and perinatal mortality and morbidity (Roberts and Lain, 2002). At present, the only available therapeutic option is removal of placenta. However, at an early gestational age, this constitutes a risk for the growth of newborn. There is a paucity of data on the etiology and the molecular basis of PE. Endothelial dysfunction, inflammation, coagulopathy, and other factors have been shown to affect the development of PE. The condition has been called as a 'disease of theories' as no single definitive cause has been identified for its etiology (Ueki et al., 2015). The paucity of data on disease pathogenesis encourages studies on the development of diagnostic markers to predict the onset of the disease which pave way for the curative methods.

Familial clustering of PE has been well documented and this indicates a genetic component for its pathogenesis. A family history of PE in a primigravida, increases the risk of severe PE by four times (Cincotta and Brennecke, 1998). Also, twin studies have estimated the heritability of PE to be about 55% (Williams and Broughton, 2011). Both candidate gene and Genome Wide Association Screening (GWAS) approaches has been applied to understand the genetic underpinnings (Williams and Broughton, 2011; Johnson et al., 2012). Candidate gene approach has relied on single nucleotide polymorphisms (SNPs) in the genes involved in the disease's pathophysiological pathways like vasoactive proteins (*AGT*, *ACE*), thrombophilia (*F5*, *F2*, *MTHFR*, *SERPINE1*, *GPIIIA*), oxidative stress (*APOE*, *GST*), endothelial function (*VEGF*, *VEGFR1*, *eNOS3*) and immunogenetics (*TNF-α*, *IL-10*).

Hypertension is one of the cardinal signs of PE along with proteinuria. Renin-angiotensin system that regulates blood pressure is therefore considered to be a promising candidate for identifying genomic markers for PE. Angiotensin II, a potent vasoconstrictive peptide, is

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produced in the inactive form as angiotensinogen (*AGT*). A number of polymorphisms have been reported in the genes involved in the renin-angiotensin pathway. T>C polymorphism at residue 1311 in the cDNA (NM_000029.3) results in a methionine to threonine switch in the protein sequence at amino acid residue 235. The threonine variant is associated with higher plasma levels of AGT (Jeunemaitre et al., 1992). The threonine variant has also been associated with essential hypertension. Positive association between *AGT* M235T (rs 699) and PE has been reported in a meta-analysis of various studies (Lin et al., 2012). However, two independent studies from geographically distinct centres in North India found results that were contrary to the conclusions of the meta-analysis. One study failed to find any association between the threonine variant of *AGT* and PE (Aggarwal et al., 2011). Another study found a protective role for the TT genotype (Aggarwal et al., 2010). Thus the role of M235T in increasing the risk for PE in the Indian population is still not clear. Furthermore, there is no data on the status of *AGT* M235T polymorphism among preeclamptic pregnancies in the South Indian population. The conflicting results in the North Indian studies and the lack of data from the South Indian population encouraged us to undertake this study.

2. Material and methods

2.1. Study design and participants

A prospective case-control design was adopted for the study. A total of 150 preeclamptic and an equal number of normotensive pregnant women were enrolled in the *AGT* study. Participants were enrolled from the Department of Obstetrics and Gynaecology of the Medical College associated with the first author during Aug 2014 to July 2015. The study was approved by the Institutional Ethics Committee. Informed consent was obtained from each participant before enrolment.

Preeclampsia was diagnosed in pregnant women upon fulfilment of the following clinical parameters: (1) blood pressure ≥ 140/90 mmHg, (2) ≥ 20 weeks of gestation and (3) proteinuria (urinary protein ≥1 + on dip stick). Both primigravida and multigravida in the age group 20–45 years with clinically diagnosed PE and gestational age of ≥20 weeks were included in the study. Pregnant women with eclampsia, chronic hypertension, and pregnant woman <20 weeks of gestation were excluded from the study. Normotensive pregnant women who had no complication till delivery, no history of hypertension, PE or eclampsia were included as control.

2.2. Sample collection and DNA isolation

About 3 mL of peripheral venous blood was collected in EDTA vacutainer and stored at 4 °C. DNA was isolated from the peripheral blood lymphocytes using salting out method (Miller et al., 1988). Purity of the sample was determined By UV spectrophotometry (Perkin Elmer model Lambda 35, USA). Purified DNA samples were stored at −20 °C.

2.3. Genotyping AGT M235T

Genomic DNA was amplified by polymerase chain reaction on Bio Rad C1000 Touch Thermal Cycler. The primer pairs used were 5′GATGCGCACACAAGGTCCTGTC3′ (forward) 5′ CAGGGTGCTCCACACTGGACCCC 3′ (reverse). 20 μL reaction mixture included 1× assay buffer, 400 ng genomic DNA, 0.2 mM dNTP, 1 picomole of each primer, 1.5 mM $MgCl_2$ and 1 unit *Taq* DNA polymerase (Bangalore Genei). The program comprised of an initial denaturation at 95 °C for 5 min followed by 40 cycles at 95 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s; final extension involved 10 min at 72 °C. The PCR product was analysed on 2% agarose gel. The 303 bp amplicon was subjected to restriction digestion by incubating with 5 units of *Tth111I* (New England Biolabs, USA) at 65 °C overnight. The reaction mixture was analysed on 3% agarose gel with ethidium bromide staining. M235 allele was visible as an uncut 303 bp fragment while the T235 allele is cleaved to produce a 279 and 24 bp fragments. The 24 bp fragment was not visible on the gel due to its small size.

2.4. Statistical analysis

Sample size was calculated using the OpenEpi web tool considering 95% confidence interval and 80% power [www.OpenEpi.com, updated April 04, 2013, accessed May 23, 2016]. Statistical analysis was done using the Statistical Packages for Social Sciences software. (SPSS, Windows version release 13, SPSS Inc., Chicago, Illinois, USA). Allele and genotype frequencies of the two groups was compared using relevant contingency tables. Difference between the groups was determined by using Fisher's exact test. *P*-values <0.05 were considered as statistically significant. All statistical tests were two-tailed. The study population was tested for conformity to Hardy-Weinberg equilibrium using a web program (Rodriguez et al., 2009).

3. Results

Clinical parameters of the study population are given in Table 1. 60.7% of PE pregnancies were primigravida and 39.3% were multigravida. This is consistent with the trend of PE being more common among primigravida than multigravida. Mild form of PE women (62%) was more common than severe form (38%).

The distribution of M and T alleles among cases and controls is depicted in Table 2. The prevalence of T allele was higher in cases (72.0%) than in controls (60.3%). The difference in the allele distribution between the two groups was statistically significant ($P = 0.003$). The odds ratio for the occurrence of T allele in cases was 1.69 (95% CI: 1.20–2.38). The odds ratio did not change significantly when the study population was stratified into severe and mild forms (Table 3).

The profile of *AGT* M235T polymorphism in the two groups is shown in Table 2. The distribution of the genotype in both the groups was in agreement with Hardy-Weinberg equilibrium. The MM genotype was less common in cases (8.7%) than in controls (19.3%). The TT genotype was relatively more common in cases (52.7%) than in controls (40%). The difference in the profile of genotype of the two groups was statistically significant ($P = 0.012$). Table 3 shows the statistical evaluation of the genotypes in dominant and recessive genetic models. The odds ratio for the onset of PE was 2.52 (95% CI: 1.25–5.07) and 1.67 (95% CI: 1.05–

Table 1
Clinical profile of preeclampsia in the AGT study case group.

Clinical parameter	Observation ($n = 150$)
Age (years)	
- Case	24.2 ± 4.1
- Control	25.2 ± 3.7
Gravida	
- Case	Primigravida: 60.7%, Multigravida: 39.3%
- Control	Primigravida: 40.0%, Multigravida: 60.0%
Gestation (weeks)	
- Case	35.4 ± 2.0
- Control	38.0 ± 1.4
Blood pressure (mm Hg) (Systolic/Diastolic)	
- Control	111.7 ± 6.9/75.3 ± 4.4
- Mild PE	141.3 ± 11.6/94.7 ± 5.8
- Severe PE	170.0 ± 18.5/112.4 ± 5.6
Severity of PE	
- Mild	93 (62%)
- Severe	57 (38%)
Fetal distress[a]	25 (16.6%)
Intrauterine growth restriction[a]	11 (7.3%)

[a] Seen only in cases.

Table 2
Distribution of *AGT* M235T allele and genotypes in the study groups.

AGT M235T genotype/allele	Preeclamptic subjects	Normotensive subjects	*P* value
M	84	119	0.003
T	216	181	
MM	13	29	0.012
MT	58	61	
TT	79	60	

2.63) in the dominant and recessive models for the T allele respectively. Statistical testing was also performed by considering severe and mild forms separately. Results are show in Table 3. Severe PE showed significant association with TT genotype only in the dominant model. The association of mild PE with TT was seen both with dominant and recessive model. In the dominant model, the association was stronger with severe PE (OR = 3.29) than that with mild form (OR = 2.24).

4. Discussion

We examined the profile of *AGT* M235T polymorphism among PE and normotensive pregnancies in a South Indian population. Prior to this study, there were two studies from North India examining the association between PE and *AGT* M235T polymorphism (Aggarwal et al., 2011; Aggarwal et al., 2010) To the best our knowledge this is the first study to examine the involvement of *AGT* M235T polymorphism with the increased risk of developing PE in the South Indian population.

The results presented in this study shows a statistically significant association between *AGT* 235TT genotype and the increased risk of developing PE. The association between 235T and increased risk of PE was first demonstrated in 1993 in the Caucasian and Japanese populations (Ward et al., 1993). Since then the genotypic profile of *AGT* M235T polymorphism has been studied in African, Chinese, Indonesian, Sinhalese, Hispanic and Mexican populations. Both presence and absence of association between TT genotype and PE has been reported. The data available from various global centres have been subjected to a meta-analysis (Lin et al., 2012). Higher risk for the development of PE was found for the 235T allele carriers in both dominant and recessive models. When stratified by ethnicity, statistically significant association was evident in case of Caucasians, Mongolians and the total population but not in Africans. Depending on the genetic model and the study population, odds ratio ranged from 1.63 to 1.32.

There are two studies that report the status of *AGT* M235T polymorphism in the North Indian population. The first study was based in Chandigarh (Aggarwal et al., 2010) while the second study was based in Lucknow (Aggarwal et al., 2011; Aggarwal et al., 2010). However, the studies conducted in two separate centres in North India obtained results contrary to the conclusions of the meta-analysis. Furthermore, there was discordance between the results of the two studies despite being carried-out in the same population. The Chandigarh study found an association between *AGT* polymorphism and PE. However, the prevalence of TT genotype was lesser among PE subjects than among normotensive subjects. Therefore, the TT genotype was paradoxically found to be protective i.e., reduced the risk of developing PE [OR = 0.33, $P = 0.00005$ for T allele in recessive mode]. Data from this study was subsequently subjected to reanalysis during a meta-analysis (Lin et al., 2012). It was found that the distribution of the genotypes in the study population, particularly in the control set, was not in agreement with Hardy-Weinberg equilibrium. The results from the Lucknow centre were contrasting as the authors failed to find any significant association between *AGT* polymorphism and PE [OR = 1.15, $P = 0.35$ for the T allele].

Table 3
Statistical evaluation of *AGT* M235T allele and genotype association with PE.

Model	Overall	Severe PE	Mild PE
Allele	$P = 0.003$	$P = 0.024$	$P = 0.011$
T vs. M	OR = 1.69 (95% CI: 1.20–2.38)	OR = 1.71 (95% CI: 1.07–2.72)	OR = 1.69 (95% CI: 1.14–2.51)
Dominant	$P = 0.012$	$P = 0.033$	$P = 0.047$
TT + MT vs. MM	OR = 2.52 (95% CI: 1.25–5.07)	OR = 3.29 (95% CI: 1.10–9.83)	OR = 2.24 (95% CI: 1.01–4.96)
Recessive	$P = 0.036$	$P = 0.12$	$P = 0.046$
TT vs. MM + MT	OR = 1.67 (95% CI: 1.05–2.63)	OR = 1.66 (95% CI: 0.90–3.04)	OR = 1.74 (95% CI: 1.03–2.94)

Parenthesis represents 95% confidence interval for the respective OR (odds ratio).

The discordance between the two studies in the North Indian population, and also the discordance of the foresaid studies with the conclusions of the meta-analysis were part of the motivation for this study. Though meta-analyses are informative due to the pooling of data from large number of samples, they suffer from publication bias against negative data. The statistical tests used to check publication bias in meta-analysis suffer from want of sufficient power and thus their uses do not guarantee the veracity of the conclusions (Kicinski, 2014). Deviations from meta-analysis therefore merit empirical and confirmatory testing. This study is an effort in such a direction in the Indian population.

The results obtained in this study provides evidence in support of the hypothesis that *AGT* 235T allele is associated with the increased risk of developing PE in the South Indian population. Understanding the mechanism by which genes are involved in PE will enable identification of women who are at high risk. This will facilitate specialised antenatal care to this group. Knowledge of the genetic component of PE may also enable identification of novel pharmaceutical targets.

Disclosure statement

None of the authors have any conflict of interest.

References

Aggarwal, P.K., Jain, V., Jha, V., 2010. Endothelial nitric oxide synthase, angiotensin-converting enzyme and angiotensinogen gene polymorphism in hypertensive disorders of pregnancy. Hypertens. Res. 33, 473–477.

Aggarwal, S., Dimri, N., Tandon, I., Agrawal, S., 2011. Preeclampsia in North Indian women: the contribution of genetic polymorphisms. J. Obstet. Gynaecol. Res. 37, 1335–1341.

Agrawal, S., Walia, G.K., 2014. Prevalence and risk factors for symptoms suggestive of pre-eclampsia in Indian women. J. Women's Health Issues Care 3, 6.

Cincotta, R.B., Brennecke, S.P., 1998. Family history of pre-eclampsia as a predictor for pre-eclampsia in primigravidas. Int. J. Gynecol. Obstet. 60, 23–27.

Jeunemaitre, X., Soubrier, F., Kotelevtsev, Y.V., Lifton, R.P., Williams, C.S., Charru, A., et al., 1992. Molecular basis of human hypertension: role of angiotensinogen. Cell 71, 169–180.

Johnson, M.P., Brennecke, S.P., East, C.E., Göring, H.H., Kent, J.W., Dyer, T.D., et al., 2012. Genome – wide association scan identifies a risk locus for preeclampsia on 2q14, near the inhibin, beta B gene. PLoS One http://dx.doi.org/10.1371/journal.pone.0033666.

Kicinski, M., 2014. How does under-reporting of negative and inconclusive results affect the false-positive rate in meta-analysis? A simulation study. BMJ Open http://dx.doi.org/10.1136/bmjopen-2014-004831.

Lin, R., Lei, Y., Yuan, Z., Ju, H., Li, D., 2012. Angiotensinogen gene M235T and T174M polymorphisms an susceptibility of pre-eclampsia: a meta-analysis. Ann. Hum. Genet. 76, 377–386.

Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 1215.

Roberts, J.M., Lain, K.Y., 2002. Recent insights into the pathogenesis of pre-eclampsia. Placenta 23, 359–372.

Rodriguez, S., Gaunt, T.R., Day, I.N., 2009. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am. J. Epidemiol. 169, 505–514.

Ueki, N., Takeda, S., Koya, D., Kanasaki, K., 2015. The relevance of the renin-angiotensin system in the development of drugs to combat preeclampsia. Int. J. Endocrinol. http://dx.doi.org/10.1155/2015/572713.

Ward, K., Hata, A., Jeunemaitre, X., Helin, C., Nelson, L., Namikawa, C., et al., 1993. A molecular variant of angiotensinogen associated with preeclampsia. Nat. Genet. 4, 59–61.

Williams, P.J., Broughton, P.F., 2011. The genetics of pre-eclampsia and other hypertensive disorders of pregnancy. Best Pract. Res. Clin. Obstet. Gynaecol. 25, 405–417.