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# Transforming growth factor beta 1 related gene polymorphisms in gestational hypertension and preeclampsia: A case-control candidate gene association study

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ABSTRACT

*Objectives:* To investigate the association between transforming growth factor beta 1 related gene polymorphisms and the occurrence of pregnancy induced hypertension, including gestational hypertension and preeclampsia.

*Study design:* This is a case control candidate gene association study. A total of 130 patients with preeclampsia, 67 with gestational hypertension, and 316 controls were recruited. Eleven candidate SNPs of were examined, including four TGFβ1 SNPs (rs1800469, rs4803455, rs4803457, and rs8179181), one TGFβR1 SNP (rs10739778), four TGFβR2 SNPs (rs1346907, rs3087465, rs6550005, and rs877572), and two ENG SNPs (rs10121110 and rs11792480).

*Main outcome measures:* Logistic regression was used to estimate the OR and the 95% CI under different genetic models. Chi-square test was used to determine whether haplotype frequency distributions differed between the case and control groups and logistic regression was used to estimate ORs. To explore the associations between SNPs and blood pressure, SBP/DBP in different genotype groups in healthy women were compared by Wilcoxon rank sum test.

*Results:* We did not find significant association between investigated SNPs and pregnancy induced hypertension. The C-T-A-G haplotype of four TGFβR2 SNPs (rs877572, rs1346907, rs3087465, and rs6550005) was significantly associated with preeclampsia (OR = 4.71, 95%CI: 1.944, 11.409) and gestational hypertension (OR = 3.157, 95%CI: 1.003, 9.938). The AA genotype of TGFβR1 rs10739778 was found to be associated with higher blood pressure in healthy women ($p \leq .008$).

*Conclusions:* Polymorphisms in TGFβR2 might participate in the pathologic process of pregnancy induced hypertension, and TGFβR1 rs10739778 was associated with blood pressure in our healthy participants.

## 1. Introduction

The typical pathological and physiological changes of pregnancy-induced hypertension (PIH, including preeclampsia (PE) and gestational hypertension (GH)) are abnormal vascular responses to placentation, such as increased systematic vascular resistance, enhanced platelet aggregation, activation of the coagulation system, and endothelial cell dysfunction [1].

The transforming growth factor beta (TGF-β) family is a group of structures-related cytokines that regulate cell growth and differentiation [2]. TGF-β has four isoforms: TGF-β 1 to 4. As reported, TGF-β1 is associated with the pathologic processes of many diseases, including apoptosis of vascular endothelial cells, immune adaptation, embryonic growth and development [3–5]. Many evidences indicate that TGF-β1 might participate in the pathogenesis of PE, possibly through activation of endothelial cells and regulation of systemic inflammation [7,8].

Several SNPs (869T > C, 509 C > T, 800G > A, 915 G > C) related to the TGF-β1 have been investigated for their possible associations with the risk of PE. Since the results of those studies were inconsistent, we had conducted a *meta*-analysis [6]. And the pooled results showed that the TT genotype of the 869T > C was negatively associated with the risk of PE (pooled OR = 0.73, 95% CI: 0.56, 0.95) [6]. However, the number of studies on this SNP and PE was small, and our *meta*-analysis had only included four articles. Therefore, we think it

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**Table 1**
Demographic and clinical characteristics of the study subjects.

| Characteristics[a] | Controls | GH | | PE | | p[*,e] |
|---|---|---|---|---|---|---|
| | N = 316 | N = 67 | p[*,c] | N = 130 | p[*,d] | |
| Maternal age, years (M (QL, QU))[b] | 25 (23, 26) | 27 (25.0, 31.5) | < .0001 | 26 (24.0, 30.0) | < .0001 | .1543 |
| Gestational age at delivery, weeks (M (QL, QU))[b] | 39 (38, 40) | 39 (38, 40) | .1286 | 38 (37, 39) | < .0001 | .0449 |
| Cesarean section (n/N (%)) | 101/306 (33.1) | 35/52 (67.31) | < .0001 | 77/123 (62.6) | < .0001 | .5534 |
| New born weight, kg (M (QL,QU))[b] | 3.3 (3, 3.6) | 3.35 (3.10, 3.75) | .1892 | 3.10 (2.70, 3.60) | .0164 | .0151 |
| In-hospital SBP, mm Hg (M (QL, QU))[b,f] | 110 (100, 120) | 140 (130, 145) | < .0001 | 132 (140, 147) | < .0001 | .2748 |
| In-hospital DBP, mm Hg (M (QL, QU))[b,f] | 70 (60, 75) | 90 (85, 95) | < .0001 | 90 (80, 95) | < .0001 | .8955 |
| In-hospital proteinuria [g] | | | | | | |
| Urinary protein dip sticks (n/N (%)) | | | | | | |
| – | 230/274 (88.94) | 28/36 (77.78) | | 40/111 (36.04) | | |
| + | 32/274 (11.68) | 8/36 (22.22) | | 36/111 (32.43) | | |
| + + | 10/274 (3.65) | 0 | | 18/111 (16.22) | | |
| + + + | 2/274 (0.73) | 0 | | 17/111 (15.32) | | |
| 24-h urine sample, mg (M (QL,QU))[b] | 442.5 (376.25, 664.5) | 180 (130, 250) | | 661 (451, 1155) | | |
| GDM (n/N (%)) | 10/316 (3.16) | 8/67 (11.94) | .0059 | 12/130 (9.52) | .0072 | .9153 |
| Fetal sex, male (n/N (%)) | 165/305 (54.10) | 26/50 (52.00) | .7820 | 64/121 (52.89) | .8219 | .5508 |

Abbreviations: M (QL, QU, Median (lower quartile (25%), upper quartile (75%); PE, preeclampsia; GH, gestational hypertension; GDM, gestational diabetes mellitus.

[*] p values estimated from Wilcoxon rank sum test, Chi-square test or Fisher exact test.; α set at 0.0167 for multiple comparisons (Bonferroni correction, 0.05/3).

[a] Demographic and clinical characteristics of this population have also been reported in [13].

[b] For variables that did not follow a normal distribution, median and quartiles are used for the statistical description.

[c] Comparison of GH and control group.

[d] Comparison of PE and control group.

[e] Comparison of GH and PE group.

[f] SBP and DBP: blood pressure measured after women arrived to the hospital for delivery and before entering the delivery room, respectively. PE women might have received treatment to control their blood pressure before the blood pressure measurements.

[g] Proteinuria was measured by urinary protein dip sticks and/or quantitative test of 24-h urinary protein after women arrived to the hospital for delivery. The no. of women reported 24-h urinary protein level for control group, GH group, and PE group were 4, 22, and 103, respectively. Between group comparisons for proteinuria level were not applicable since different test methods have been used.

necessary to validate the association between 869T > C and PE in other populations.

Endoglin is a co-receptor for TGF-β1 and TGF-β3 [7]. Soluble endoglin (sENG) is found to be up-regulated in preeclampsia and the serum level of sENG is significantly associated with the severity of PE [8]. sENG is an anti-angiogenic protein that may bind to circulating TGF-β1 and inhibit its signaling in vasculature [7]. Genes associated with this signaling pathway have been regarded as the candidate genes related to PE. Bell et al. analyzed 49 TGF and ENG related SNPs, and found ENG rs10121110 and rs11792480, TGF β receptor 1(TGFβR1) rs10739778, and TGF β receptor 2 (TGFβR2) rs6550005, rs1346907, rs3087465 and rs877572 were significantly associated with the risk of PE [9]. However, this is a single site study with limited sample size, therefore validation studies are needed. Besides, this study reported the associations of these SNPs with PE, but had not investigated their associations with GH. Given these reasons, we aim to explore the possible associations of these SNPs with PE and GH in a Chinese population.

## 2. Methods

### 2.1. Study population

A total of 130 patients with PE, 67 with GH, and 316 controls were recruited from the Liuyang Municipal Hospital of Maternal and Child Health, Hunan Province of China [10]. The inclusion criteria for the case group were clinical diagnosis of GH or PE combined with the absence of diabetes mellitus, renal disease, CVDs, or other diseases that are already known as risk factors for GH and PE. The inclusion criteria for the control group was normotensive pregnant women without aforementioned diseases who delivered at the same hospital during the same period. All of the subjects provided written informed consent, after which blood samples and medical records were collected. The study protocol was reviewed and approved by the Central South University's Ethical and Confidentiality Committee.

### 2.2. Diagnostic criteria

The diagnostic criteria for PE was *de novo* hypertension (systolic blood pressure ≥140 mm Hg and/or diastolic 90 mm Hg) after 20 weeks of gestation accompanied by proteinuria (urinary protein dip sticks ≥2+ or ≥300 mg in a 24-h urine sample) [11]. And GH was defined as *de novo* hypertension, but without the presence of proteinuria [11].

### 2.3. SNP selection and genotyping

Eleven candidate SNPs of were selected, including four TGFβ1 SNPs (rs1800469, rs4803455, rs4803457, and rs8179181), one TGFβR1 SNP (rs10739778), four TGFβR2 SNPs (rs1346907, rs3087465, rs6550005, and rs877572), and two ENG SNPs (rs10121110 and rs11792480).

Genomic DNA was extracted from whole blood using the TIANamp Blood DNA Kit (DP318-03, TIANGEN, Beijing). SNPs were genotyped with the SEQUENOM MassARRAY iPLEX platform [12].

### 2.4. Statistical analysis

Demographic and clinical features of case and control groups were compared by *t*-test or Wilcoxon rank sum test for continuous variables, and the Chi-square test was used for categorical variables. The Bonferroni correction was applied for multiple comparisons (α = 0.05/3 = 0.0167). The Chi-square goodness-of-fit test or the Fisher's exact test was used for the Hardy–Weinberg test (α = 0.01). The Chi-square test was used to test the genotype distribution between the case and control groups. A p-value of 0.05 was used to identify potential correlations for purpose of exploration. Logistic regression was used to estimate the OR and the 95% CI under different genetic models. Chi-square tests were used to determine whether haplotype frequency distributions differed between the case and control groups and logistic regression was used to estimate ORs. To explore the associations between SNPs and blood pressure, SBP/DBP in different genotype groups