Association between RUNX3 gene polymorphisms in severe preeclampsia and its clinical features

Yanping Zhang, MSa, Tao Wang, PhDb, Jin Jia, MDa, Wen Cao, MSa, Lei Ye, MSa, Yanyun Wang, PhDb, Bin Zhou, PhDb, Rong Zhou, MDa,∗

1. Introduction
Preeclampsia is a life-threatening syndrome, affecting 5% to 8% of pregnancies.[1] It is the leading cause of perinatal morbidity and mortality worldwide. Furthermore, offspring from females that suffered preeclampsia exhibit an elevated risk for diabetes, stroke, cardiovascular, and mental disorders in the short and long term.[2] The pathogenesis of preeclampsia remains largely unknown and thereby no effective methods for the prevention and treatment are available except delivery.[3] Placental insufficiency is generally considered as the major driving force in the onset of preeclampsia and the process is primarily triggered by inadequate trophoblast invasion.[3] In early placental development, trophoblasts are considered as “pseudo-tumor cells” and share certain similar mechanisms with malignant cells in tumorigenesis and metastasis.[6,7] It is worth noting that genetic factors are believed to be involved in the development of severe preeclampsia (SPE).[8,9]

Human runt-related transcription factor 3 (RUNX3), a newly discovered tumor suppressor gene, has been studied in a wide spectrum of invasive or preinvasive epithelial and mesenchymal neoplasms.[10] The RUNX3 gene is located on chromosome 1p36.1 and its overall size is ∼67 kb, containing 6 exons and 5 introns.[11] Various single nucleotide polymorphisms (SNPs) occur on the RUNX3 gene,[12] which have been associated with the risk of systemic lupus erythematosus, ankylosing spondylitis, and juvenile idiopathic arthritis.[13] Recently, 3 SNPs (rs2236852, rs7528484, and rs760805) on the RUNX3 gene have been associated with the susceptibility of certain cancers, including colorectal, gastric, and bladder cancers.[12,16,17] RUNX3 serves critical roles in the regulation of invasion, migration, and proliferation of malignant cells and its expression is strongly associated with tumorigenesis and metastasis.[10] A previous

Abstract
Preeclampsia is a complex genetic disorder and its pathogenesis remains to be investigated. Single nucleotide polymorphisms serve important roles in genetic predisposition. The present study aimed to explore the association between runt-related transcription factor 3 (RUNX3) gene polymorphisms in severe preeclampsia (SPE) and clinical features.

A total of 417 participants were enrolled in the present study. The rs2236852, rs7528484 and rs760805 polymorphisms of the RUNX3 gene were tested using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Clinical information of patients and controls was collected. Relationship between clinical feature and different genotype was analyzed.

Compared with rs2236852 GG genotype carriers, the odds ratios (OR) for the risk of SPE were 2.26 [95% confidence interval (CI), 1.24–4.12; P = .023] in AA genotype carriers. A significantly increased risk of SPE was determined to be associated with AG/AA genotypes compared with GG genotypes (OR, 1.74; 95% CI, 1.11–2.75; P = .015). AA homozygote carriers with SPE exhibited lower birth weight, shorter birth length and reduced incidence of hypoproteinemia compared with AG heterozygote carriers (P < .05). A significantly increased risk of SPE was determined to be associated with the rs7528484 CC genotype in codominant and recessive models (CC vs TT; OR, 3.70, 95% CI, 1.31–10.43, P = .01; CC vs TT/TC; OR, 3.98, 95% CI, 1.46–10.87, P = .003). Patients carrying C-allele (TC + CC) presented increased systolic pressure and an increased incidence of neonatal pneumonia compared with TT homozygote carriers (P < .05). Compared with rs760805 TT homozygote carriers, patients carrying AA homozygote exhibited significantly reduced 24 hours urinary protein levels, lower serum creatinine concentrations and a decreased incidence of neonatal asphyxia (P < .05).

The present study suggested a genetic association between RUNX3 gene polymorphisms and SPE. The data provided a novel insight to guide future investigations.

Abbreviations: CI = confidence interval, OR = odds ratio, RUNX3 = human runt-related transcription factor 3, SNP = single nucleotide polymorphism, SPE = severe preeclampsia.

Keywords: human runt-related transcription factor 3, polymorphisms, severe preeclampsia
study has demonstrated that RUNX3 is expressed in the uterus and high levels of RUNX3 at the maternal-fetal interface are beneficial to trophoblast invasion and embryo implantation.[15] Recently, an epigenetic study has indicated that the placental RUNX3 methylation status is positively correlated with plasma F/E (cortisol/cortisone) levels and neonatal birth weight of patients with preeclampsia.[16] As the invasion process of trophoblasts is similar to that of malignant cells and there may be a potential association between placental RUNX3 epigenetic modification and preeclampsia, it is speculated here that RUNX3 may be involved in preeclampsia.[6] However, the association between the rs2236852, rs7528484, and rs760805 SNPs and SPE has not been reported previously. The present study aimed to investigate the association between these SNPs on the RUNX3 gene in SPE and clinical features in Southwestern Chinese population.

2. Materials and methods

2.1. Study subjects

The present case-control study was approved by the Ethics Committee of West China Second University Hospital, Sichuan University. All participants provided written informed consent. A total of 417 participants, including 206 normal pregnancies, 100 patients with early-onset and 111 patients with late-onset SPE were recruited from West China Second University Hospital between September 2014 and March 2016. SPE was defined as systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥110 mmHg on at least 2 occasions following 20 weeks of gestation, along with significant proteinuria (>2 g/24h and/or ≥2+ on dipstick testing) or evidence of multiorgan problems, including pulmonary edema, seizures, oliguria, thrombocytopenia, liver dysfunction or central nervous system perturbations.[20] Early-onset preeclampsia was defined as manifestation before 34 gestational weeks and late-onset preeclampsia thereafter.[21] Normal pregnant women were confirmed without uncomplicated symptoms of gestational hypertension and proteinuria during the same period. Exclusion criteria included diabetes, heart diseases, chronic hypertension, kidney diseases, autoimmune diseases, thrombophilia, multiple pregnancy, and fetal malformation. Gestational age was confirmed by routine ultrasound measurements in the first trimester.

2.2. DNA extraction and genotyping

Three SNPs (rs2236852, rs7528484, and rs760805) in RUNX3 gene were genotyped in the present study. Genomic DNA was extracted from 2 mL EDTA-anticoagulated peripheral blood using a DNA extraction kit (BioTeke, China) according to the manufacturer’s instructions. All polymerase chain reaction (PCR) amplifications were performed in a final volume of 10 μL, containing 3.8 μL ultrapure water, 5 μL 2×Power Taq PCR Master mix (BioTeke, China), 0.5 mmol/L primer and 100 ng genomic DNA. The investigated DNA sequences were amplified using the following primers: rs2236852, forward, 5’-TGGAGTGCTCCTCCCTTT CTTG-3’ and reverse, 5’-TATGGCAGGCTGGGACACCTC-3’ (120 bp product); rs7528484, forward, 5’-CCGAGCGCCAGGGGTTG TA-3’ and reverse, 5’-CATGGGAAGGGCACCCTTCGGTG AGGTA-3’ (125 bp product); and rs760805, forward, 5’-TCTCCACATCAAGGGTCCAG-3’ and reverse, 5’-TACAGCTCTCAATATGC CCAG-3’ (174 bp product).

The PCR temperature protocol followed an initial denaturation at 94°C for 4 minutes, followed by 34 circles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 63.6°C (rs2236852), 60°C (rs7528484) or 58.7°C (rs760805) and a final elongation at 72°C for 10 minutes. PCR products were digested using NdeI (rs2236852), HincII (rs7528484), or BstZI7I (rs760805) at 37°C for 2 hours in a 10 μL reaction volume.

2.3. Restriction-fragment length polymorphism analysis

Digested products were separated by a 6% polyacrylamide gel and stained with 1.5 g/L argent nitrate. Emerging bands and corresponding genotypes were as follows: i) rs2236852 locus: Genotype AA, 100bp; AG, 120 and 100bp; GG, 120bp; ii) rs7528484 locus: Genotype CC, 107bp; TC, 125 and 107bp; TT, 125bp; and iii) rs760805 locus: Genotype AA, 152bp; AT, 174 and 152bp; TT, 174bp (Fig. 1).

Samples (10%) were selected at random for repeated analysis and results were 100% concordant.

2.4. Statistical analysis

Data were analyzed using SPSS 19.0 (SPSS Inc, Chicago, IL). Continuous data are presented as the mean±standard deviation and categorical variables are expressed using percentages Genotype and allele frequencies of the SNPs were obtained by direct computing. Genotypic association tests in a case–control pattern, assuming codominant, dominant, recessive, overdominant, or log-additive genetic models were performed using SNPstats online software (www.snpstats.net/start.htm).[22] Odds ratio (OR) with 95% confidence intervals (CI) were determined to estimate the relative risk for preeclampsia. Clinical characteristics among various genotype (rs2236852 and rs760805) polymorphisms were evaluated using one-way analysis of variance (ANOVA) and Student t tests were used to analyze clinical characteristics between various genotypes of rs7528484 polymorphisms. Chi-square tests were performed to evaluate differences in clinical outcomes. P<.05 was considered to indicate a statistically significant difference.

3. Results

3.1. Baseline characteristics

Patient characteristics are presented in Supplementary Table S1 (see Table S1, http://links.lww.com/MD/C889). No significant difference was observed in maternal age, early pregnancy body mass index, gravidity, parity, and proportion of smokers among the 3 groups (P>.05). A significant difference was determined in gestational age at sampling between the early- and the late-onset group (P<.001). A significant difference was determined in gestational age at sampling between the early- and the late-onset group (P<.001; Table S1, http://links.lww.com/MD/C889).

3.2. Distribution of genotype frequencies

Genotype distributions of the polymorphisms were consistent with the Hardy-Weinberg equilibrium. Compared with rs2236852 GG genotype carriers, the OR for the risk of SPE was 2.26 (95% CI, 1.24–4.12; P=.023) in AA genotype carriers. Significantly increased risk of SPE was further associated with AG/AA genotypes compared with GG genotypes (OR, 1.74; 95%
Significantly increased risk of SPE was determined to be associated with rs7528484 CC genotype in codominant and recessive models (CC vs TT: OR, 3.70; 95% CI, 1.31–10.43; P = .01; CC vs TT/TC: OR, 3.98; 95% CI, 1.46–10.87; P = .003). No significant differences were detected in genotype frequencies of the rs760805 polymorphism between patients with SPE and the control (P > .05; Table 1).

As presented in Table 2, an increased risk of late-onset SPE was associated with the AA/AG genotypes of the rs2236852 polymorphism in a dominant model compared with GG genotype (OR, 1.80; 95% CI, 1.03–3.16; P = .035). A significantly increased risk of late-onset SPE was further determined for AA genotype carriers in a recessive model compared with GG/AG genotype carriers (OR, 1.90; 95% CI, 1.07–3.38; P = .029). Furthermore, as presented in Table 3, rs7528484 CC genotype carriers had a 3.50-fold increased risk of early-onset SPE (95% CI, 1.11–10.75; P = .015). A significantly increased risk of SPE was determined to be associated with rs7528484 CC genotype in the control group. Systolic pressure of TT homozygote carriers was decreased compared with C-allele carriers (TC+CC) in the early-onset group. Similarly, females with TT homozygote in the late-onset group exhibited a decreased blood urea nitrogen level compared with C-allele carriers (TC+CC; Table 5).

In the control group, systolic pressure of rs760805 TT genotype carriers was markedly elevated compared with AT genotype carriers. Compared with patients carrying AA genotype, TT genotype carriers presented increased neonatal birth weight, lower levels of serum creatinine and albumin. Additionally, females with the AT heterozygote were revealed to present significantly increased neonatal birth weight and reduced serum albumin level compared with AA homozygote carriers. Compared with TT homozygote carriers in the early-onset group, patients with AA homozygotes presented decreased 24-hours urinary protein and serum creatinine and additionally levels of serum creatinine were decreased in AT genotype carriers. There were no differences presented in clinical characteristics between different genotypes of the rs7528484 polymorphism in the late-onset group.
nemia compared with AA genotype carriers. Additionally, a significantly increased incidence of neonatal asphyxia was observed in AA genotype carriers compared with AG genotype carriers. As for the rs7528484 locus, the incidence of hypoproteinemia in patients with TT homozygotes was significantly decreased compared with C-allele (TC+CC) carriers, while patients with the TT genotype exhibited an increased occurrence rate of neonatal pneumonia compared with C-allele (TC+CC) carriers. Compared with patients carrying AA and AT genotypes at the rs760805 locus, TT homozygote carriers exhibited increased occurrence rates of neonatal asphyxia (Table 7). No significant differences were observed in clinical outcomes of the 3 SNPs in the late-onset group (see Table S3, http://links.lww.com/MD/C889, Supplemental Content, which clarifies the pregnancy outcomes for different genotypes of RUNX3 gene polymorphisms in the late-onset group).

### Table 1

| Genetic model | Genotype | Patients n=211, % | Control n=206, % | Logistic regression OR (95% CI) | P value |
|---------------|----------|------------------|-----------------|---------------------------------|---------|
| rs2236852     | GG       | 41 (19.4%)       | 61 (29.6%)      | 1.00 (reference)                |         |
|               | AG       | 123 (58.3%)      | 114 (55.3%)     | 1.61 (1.00–2.57)                | .023†   |
|               | AA       | 47 (22.3%)       | 31 (15.1%)      | 2.26 (1.24–4.12)                | .015†   |
| rs7528484     | GG       | 41 (19.4%)       | 61 (29.6%)      | 1.00 (reference)                |         |
|               | AG/AA    | 170 (80.6%)      | 145 (70.4%)     | 1.74 (1.11–2.73)                |         |
|               | AA       | 47 (22.3%)       | 31 (15.0%)      | 1.62 (0.98–2.67)                | .058    |
| rs760805      | GG       | 65 (30.8%)       | 63 (30.6%)      | 1.00 (reference)                |         |
|               | AT       | 111 (52.6%)      | 103 (50.0%)     | 1.04 (0.67–1.62)                | .96     |
|               | TT       | 35 (16.6%)       | 40 (19.4%)      | 0.85 (0.48–1.50)                | .35     |

CI = confidence interval, OR = odds ratio, RUNX3 = human runt-related transcription factor 3, SNP = single nucleotide polymorphism.

#### Table 2

| Genotype frequencies of rs2236852 in women with and without preeclampsia. |
|-------------------------------|------------------|------------------|------------------|------------------|
| rs2236852                     | Genetic model    | Dominant         | Recessive        | Overdominant     |
| GGA                           | GG VS AG/AA OR (95%CI) P value | GG/AG VS AA OR (95%CI) P value | GG/AA VS AG OR (95%CI) P value |
| Early-onset group             | 61 (61.0%)       | 20 (20.0%)       | 1.68 (0.95–2.99) | .069             | 1.32 (0.71–2.48) | .39   | 1.26 (0.78–2.05) | .35   |
| Late-onset group              | 62 (55.9%)       | 21 (18.9%)       | 1.80 (1.03–3.16) | .035†            | 1.90 (1.07–3.38) | .029† | 1.02 (0.64–1.62) | .93   |
| Control group                 | 114 (55.3%)      | 21 (29.6%)       | 1.00 (reference) | .94 (0.71–1.24)  | .65              |

CI = confidence interval, OR = odds ratio, SPE = severe preeclampsia.

† P<.05.
Serum albumin, g/L 38.36
24 h urine protein, g
Serum creatinine, µmol/L

Birth weight, g 3307

±

Clinical characteristics of different genotypes at rs2236852 locus.

Genotype frequencies of rs7528484 in women with and without preeclampsia. It was demonstrated that the RUNX3 rs760805 polymorphism was not associated with SPE, but rs2236852 and rs7528484 polymorphisms were identified to be involved with increased risks for preeclampsia occurrence. In addition, the various genotypes of rs2236852, rs7528484, and rs760805 polymorphisms were closely associated with clinical characteristics and pregnancy outcomes. To the

4. Discussion

Preeclampsia is a complex genetic disorder with incompletely understood pathogenesis. The present study focused on 3 SNPs and examined the association between these SNPs and SPE in the Southwestern Chinese population. It was demonstrated

Table 3

| Genotype | SPE | Dominant | Recessive | Overdominant |
|----------|-----|----------|----------|--------------|
|          | TT  | TC       | CC       | TT VS TC/CC  | OR (95%CI) | P value | TT/TC VS CC | OR (95%CI) | P value | TT/CC VS TC | OR (95%CI) | P value |
| Early-onset group | 34 (34.0%) | 58 (58.0%) | 8 (8.0%) | 1.02 (0.62–1.69) | .94 | 3.50 (1.11–10.98) | .020† | 0.81 (0.50–1.31) | .39 |
| Late-onset group | 30 (35.1%) | 61 (55%) | 11 (9.9%) | 0.97 (0.60–1.58) | .9 | 4.42 (1.50–13.07) | .005† | 0.71 (0.45–1.14) | .16 |
| Control group | 71 (34.5%) | 130 (63.1%) | 5 (2.4%) | 1.00 | .94 | 3.50 (1.11–10.98) | .020† | 0.81 (0.50–1.31) | .39 |

Table 4

Clinical characteristics of different genotypes at rs2236852 locus.

| Clinical characteristics | Control group (n = 206) | Early-onset group (n = 100) | Late-onset group (n = 111) |
|-------------------------|-------------------------|-----------------------------|----------------------------|
|                         | AA (n = 31) | AG (n = 114) | GG (n = 61) | AA (n = 19) | AG (n = 61) | GG (n = 20) | AA (n = 28) | AG (n = 62) | GG (n = 21) |
| Delivery weeks, wk       | 39.53±9.94 | 39.68±0.88 | 40.65±6.77 | 32.33±2.07 | 31.29±4.87 | 31.31±3.22 | 36.95±1.46 | 37.22±1.94 | 36.80±2.02 |
| SBP, mmHg                | 113.97±8.93 | 111.55±7.91 | 114.48±10.76 | 159.00±16.9 | 158.64±16.7 | 169.30±24.2 | 162.54±13.4 | 160.35±13.3 | 162.62±12.2 |
| DBP, mmHg                | 71.97±8.42 | 69.80±8.66 | 73.15±10.11 | 102.11±14.4 | 103.57±12.3 | 106.50±13.7 | 106.29±10.8 | 103.10±10.5 | 103.05±9.8 |
| Blood urea nitrogen, mmol/L | 3.41±1.00 | 3.29±0.95 | 3.93±2.35 | 5.45±3.44 | 5.24±2.95 | 4.75±2.16 | 4.36±1.78 | 4.74±2.03 |
| Serum creatinine, µmol/L | 43.84±7.66 | 46.47±8.56 | 45.92±7.49 | 67.58±23.60 | 64.18±21.76 | 77.65±77.31 | 66.04±24.70 | 63.00±21.7 | 61.24±19.56 |
| 24 h urine protein, µg/L | 38.68±3.71 | 38.80±3.62 | 38.32±3.51 | 31.07±4.69 | 29.00±4.63 | 31.49±4.87 | 32.01±5.10 | 32.08±4.81 | 32.20±4.42 |
| Serum albumin, g/L       | 330.37±477.5 | 322.9±398.7 | 335.5±316.9 | 1541±43.4 | 1494±72.8 | 1397±535.0 | 2330±535.2 | 2674±683.2 | 2561±904.2 |
| Birth weight, g          | 49.65±2.57 | 49.68±1.64 | 50.52±6.36 | 39.82±4.41 | 39.03±5.80 | 38.35±5.06 | 44.61±3.98 | 46.76±3.93 | 46.19±4.85 |

DBP = diastolic blood pressure at sampling, SBP = systolic blood pressure at sampling.
Data are presented as mean±SD.
AA genotype carriers compared with AG genotype carriers, P < .05.
AG genotype carriers compared with GG genotype carriers, P < .05.

Table 5

Clinical characteristics of different genotypes at rs7528484 locus.

| Clinical characteristics | Control group (n = 206) | Early-onset group (n = 100) | Late-onset group (n = 111) |
|-------------------------|-------------------------|-----------------------------|----------------------------|
|                         | TT (n = 71) | TC+CC (n = 135) | TT (n = 34) | TC+CC (n = 66) | TT (n = 39) | TC+CC (n = 72) |
| Delivery weeks, wk       | 40.59±6.28 | 39.60±0.87 | 31.85±2.49 | 31.30±4.80 | 37.06±1.77 | 37.08±1.89 |
| SBP, mmHg                | 112.49±9.67 | 112.93±10.16 | 165.24±15.7 | 163.73±19.6 | 159.85±10.6 | 162.14±14.2 |
| DBP, mmHg                | 71.03±9.46 | 71.16±9.04 | 101.15±10.5 | 105.29±13.9 | 104.36±10.1 | 103.64±10.7 |
| Blood urea nitrogen, mmol/L | 3.42±1.03 | 3.24±0.97 | 5.47±3.70 | 5.52±2.85 | 4.07±1.30 | 4.80±2.15 |
| Serum creatinine, µmol/L | 45.94±7.67 | 45.90±8.74 | 62.15±16.83 | 70.29±49.8 | 58.95±15.7 | 65.86±24.5 |
| 24 h urine protein, µg/L | 38.36±4.03 | 38.79±3.34 | 30.45±4.53 | 29.60±4.90 | 32.02±5.1 | 32.12±4.6 |
| Serum albumin, g/L       | 336.8±456.9 | 331.7±347.2 | 1569±672.5 | 1439±634.4 | 2473±590.5 | 2616±776.6 |
| Birth weight, g          | 49.70±1.88 | 50.04±4.47 | 39.13±5.22 | 38.98±5.52 | 45.56±4.1 | 46.40±4.24 |

DBP = diastolic blood pressure at sampling, SBP = systolic blood pressure at sampling.
Data are presented as mean±SD.
TT genotype carriers compared with C-allele (TC + CC) carriers, P < .05.
As the small number of CC genotype, TC and CC genotypes are combined for statistical analysis.
best of our knowledge, this is the first study to evaluate RUNX3 gene polymorphisms in SPE. It was discovered that AA and AG/AA genotypes of the rs2236852 polymorphism were more frequent in patients with SPE. The findings suggested that SNP rs2236852 may be a risk factor for SPE, which has not been reported previously. Numerous previous studies demonstrated that AA genotype carriers of the rs2236852 polymorphism were associated with a reduced risk of gastric cancer in the Chinese population and colorectal cancer in the Mexican population.\(^{1,2,3,4}\) Inconsistencies among these findings are potentially based on ethnic differences and varying disease types. The rs2236852 polymorphism is located in an intron 4 region without apparent regulatory sequences\(^{1,7}\) and studies have identified that a transcription factor binding site, which is highly correlated with the sequence fragments, is located 4 nucleotides upstream of this polymorphism.\(^{2,4}\) Therefore, the role of the rs2236852 polymorphism in SPE may be associated with changes in gene expression or a combination with an unidentified functional variant of the gene. The data indicated that AA homozygote carriers with SPE exhibited lower birth weight and shorter birth length compared with AG heterozygote carriers. This may partially be supported with the increased risk of SPE. Compared with AG heterozygote carriers with SPE, AA homozygote carriers were observed with decreased incidence of hypoproteinemia, while GG homozygote carriers exhibited increased systolic blood pressures and increased incidence of neonatal asphyxia. In the analysis of the potential reasons, the predominant amount of AG

### Table 6
Clinical characteristics of different genotypes at rs760805 locus.

| Clinical characteristics | Control group (n=206) | Early-onset group (n=100) | Late-onset group (n=111) |
|--------------------------|-----------------------|--------------------------|--------------------------|
|                          | AA (n=63) (n=63)       | AT (n=103) (n=103)       | TT (n=40) (n=40)         |
|                          | AA (n=25) (n=25)       | AT (n=56) (n=56)         | TT (n=19) (n=19)         |
|                          | AA (n=40) (n=40)       | AT (n=55) (n=55)         | TT (n=16) (n=16)         |
| Delivery weeks, wk       | 39.50±0.85            | 40.12±5.28              | 39.66±0.84               |
| SBP, mmHg                | 112.81±9.31           | 111.58±10.20            | 115.83±10.1 ±         |
| DBP, mmHg                | 71.46±8.56            | 70.16±9.24              | 73.05±0.74               |
| Blood urea nitrogen, mmol/L | 3.29±0.87           | 3.29±1.08               | 3.36±0.95               |
| Serum creatinine, μmol/L | 44.24±8.38\(^*\)      | 46.19±7.84              | 47.83±8.35               |
| Serum albumin, g/L       | -                     | -                       | 3.92±2.47\(^*\)         |
| 24 h urine protein, g    | 39.76±3.01\(^*\)      | 38.27±3.50              | 37.81±3.76               |
| Birth weight, g          | 3315±317.8\(^*\)      | 3375±427.3              | 3391±359.2              |
| Birth length, cm         | 49.59±1.53            | 49.71±1.90              | 51.03±7.78              |

Table 7
Pregnancy outcomes for different genotypes of RUNX3 gene polymorphisms in the early-onset group.

| Complications            | Early-onset group | AA (n=19) (n=19) | AG (n=61) (n=61) | GG (n=20) (n=20) |
|--------------------------|-------------------|------------------|-----------------|-----------------|
| HIEBP syndrome, n (%)    | 12 (12.00)        | 3 (15.79)        | 7 (11.48)       | 2 (10.00)       |
| Placental abruption, n (%)| 5 (5.00)          | 2 (10.53)        | 2 (3.28)        | 1 (5.00)        |
| Visual disturbance, n (%)| 30 (30.00)        | 2 (10.53)        | 23 (37.70)      | 3 (15.00)       |
| Hypoproteinemia, n (%)   | 58 (58.00)        | 7 (36.84)\(^*\)  | 41 (67.21)      | 10 (50.00)      |
| Pleurapertoneal fluid, n (%) | 28 (28.00)    | 8 (42.11)        | 13 (21.31)      | 6 (30.00)       |
| Liver dysfunction, n (%) | 41 (41.00)        | 6 (31.58)        | 25 (40.98)      | 10 (50.00)      |
| Neonatal infant death, n (%) | 32 (32.00)     | 3 (15.79)        | 22 (36.07)      | 7 (35.00)       |
| Neonatal asphyxia, n (%) | 39 (39.00)        | 10 (52.63)       | 15 (24.59)      | 14 (70.00)      |
| Neonatal pneumonia, n (%)| 31 (31.00)        | 6 (31.58)        | 15 (24.59)      | 10 (50.00)      |
| Neonatal intracranial hemorrhage, n (%) | 30 (30.00) | 4 (22.11)        | 13 (21.31)      | 9 (45.00)       |
| Neonatal hypoxiaemia, n (%) | 13 (13.00)     | 2 (10.53)        | 8 (13.11)       | 4 (20.00)       |
| Neonatal retinopathy, n (%) | 14 (14.00)     | 3 (15.79)        | 9 (16.07)       | 7 (30.36)       |

Data are presented as percentage.
\(^{1}\) AA genotype carriers compared with AG genotype carriers, P<.05.
\(^{2}\) AG genotype carriers compared with GG genotype carriers, P<.05.
\(^{3}\) rs2236852.
\(^{4}\) rs7528484.
\(^{5}\) rs760805.
\(^{6}\) TT genotype carriers compared with TC+CC carriers, P<.05.
heterozygote carriers may contribute to these results. In addition, AG or GG genotypes may be risk factors directly resulting in poor pregnancy outcomes. However, the relatively small sample size of the present study describes a limitation and should be considered in the future.

The present study provided evidence first that the CC genotype of the rs7528484 polymorphism was more frequent in females with SPE. The data were in agreement with the previous study reporting that the rs7528484 polymorphism increased genetic susceptibility to intestinal-type gastric cancer by increasing promoter activity in the Korean population.[27] Furthermore, CC-homozygote carriers with preeclampsia were more likely to suffer from clinical manifestations, including high blood pressure and increased urea nitrogen levels, and poor perinatal outcomes. The data suggested that rs7528484 may be involved in SPE. Unexpectedly, patients with the TT genotype exhibited increased occurrence rates of neonatal pneumonia compared with C-allele (TC+CC) carriers. An analysis of the potential reasons suggested that the small sample size may contribute to these unexpected results. The TT homozygote may be a risk factor negatively affecting pregnancy outcomes.

The present study did not reveal any differences in genotype frequencies of the rs760805 polymorphism among healthy females and early- or late-onset preeclampsia. Recent studies on the association between the rs760805 polymorphism and malignant diseases are controversial. Certain studies suggested that the AA genotype is associated with an increased risk of bladder and gastric cancer in varying populations.[17,23,28] Nadarajan et al. discovered no difference between the genotype and allele frequencies in the India population suggesting no association between the rs760805 polymorphism and an increased risk of gastric cancer.[16] Suárez-Villanueva et al. proposed that the genotype and allele frequencies were not significantly different in Mexican patients with colorectal cancer compared with the healthy controls.[12] However, ethnic difference and different disease types may contribute to the inconsistency of the results. Additionally, the present study provided evidence that AA homozygote carriers with preeclampsia at the rs760805 polymorphism were associated with various important clinical manifestations, including serum creatinine, and 24 hours urinary protein levels. TT homozygote carriers with preeclampsia were observed with a higher incidence of adverse reactions, particularly neonatal asphyxia. These findings indicated that the rs760805 polymorphisms may be involved in preeclampsia, but further research is needed.

There were certain limitations associated with the present study. First, the small sample size may contribute to the potential of certain statistically significant results to have occurred by chance. Furthermore, genotyping was not performed in the placenta of the recruited participants. It remains unknown whether the investigated SNPs have an impact on RUNX3 expression and the potential mechanism associated with the RUNX3 gene and its involvement in SPE remains to be explored. Therefore, further investigations are necessary to enrich and verify the presented results and to clarify the involvement of RUNX3 in SPE.

5. Conclusion

The current case-control study described the genetic association between RUNX3 rs2236852 and rs7528484 gene polymorphisms and the risk of preeclampsia. The presence of RUNX3 rs2236852 and rs7528484 variants may contribute to an increased risk of SPE. Additionally, RUNX3 gene rs2236852, rs7528484, and rs760805 polymorphisms were associated with important clinical manifestations and pregnancy outcomes. Preeclampsia is regarded as a multifactorial disease, with environmental and genetic factors potentially interfering with the presented results. Further genetic and functional multi-center and large-scale studies investigating varying ethnicities are required to validate the presented findings. Whether the increased risk of preeclampsia is associated with biochemical or functional variations of RUNX3 and associated pathways remains to be explored.

Author contributions

Conceptualization: Rong Zhou.
Data curation: Yanping Zhang, Jin Jia, Wen Cao, Lei Ye.
Formal analysis: Yanping Zhang, Jin Jia, Wen Cao, Lei Ye.
Funding acquisition: Tao Wang, Rong Zhou.
Investigation: Jin Jia, Wen Cao, Lei Ye.
Methodology: Yanping Zhang, Tao Wang, Jin Jia, Wen Cao, Lei Ye.
Project administration: Yanping Zhang, Tao Wang, Jin Jia, Wen Cao, Lei Ye, Yanyun Wang, Rong Zhou.
Resources: Yanping Zhang, Jin Jia, Wen Cao, Lei Ye, Yanyun Wang, Bin Zhou, Rong Zhou.
Software: Yanping Zhang, Tao Wang, Yanyun Wang.
Supervision: Tao Wang, Bin Zhou, Rong Zhou.
Validation: Rong Zhou.
Visualization: Tao Wang.
Writing – original draft: Yanping Zhang, Tao Wang.
Writing – review & editing: Tao Wang, Yanyun Wang, Bin Zhou, Rong Zhou.

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