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Genetic variants in *DICER1*, *DROSHA*, *RAN*, and *XPO5* genes and risk of pregnancy-induced hypertension

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ABSTRACT

Objective: To evaluate the impact of microRNA (miRNA) machinery gene polymorphisms on the risk of gestational hypertension (GH) and preeclampsia (PE).

Study design: A case-control study among Han Chinese with a total of 143 patients diagnosed with PE, 79 with GH, and 330 healthy controls was conducted. Nine candidate SNPs in 4 selected miRNA biogenesis genes were genotyped, including three *DICER1* SNPs (rs3742330, rs1057035 and rs13078), two *DROSHA* SNPs (rs17409893 and rs642321), two *RAN* SNPs (rs3803012 and rs14035), and two *XPO5* SNPs (rs1106841 and rs2257082). Logistic regression was used to estimate the odds ratios (OR) and 95% confidence intervals (CIs) for the associations.

Results: The *DICER1* rs13078 TA genotype was still strongly associated with an increased risk of GH (OR = 3.17 (95% CI: 1.55, 6.48)), and the *RAN* rs3803012 AG genotype was associated with an increased risk of PE (OR = 2.15 (1.26, 3.66)). The *RAN* rs14035/rs3803012 haplotype C-G was associated with PE susceptibility (OR = 2.08 (95% CI: 1.19, 3.62)); however, the haplotype C-A was a protective factor for PE (OR = 0.68 (95% CI: 0.50, 0.93)). The *DICER1* rs1057035/ rs13078/ rs3742330 haplotype T-A-A and *DORSHA* rs17409893/ rs642321 haplotype A-T were both associated with increased risk of GH (OR = 2.75 (95% CI:1.40, 5.38) and OR = 1.60 (95% CI:1.12, 2.29), respectively). These significant associations were retained after false-positive discovery rate correction ($p < 0.05$).

Conclusions: Genetic variants in miRNA machinery genes might participate in the development of pregnancy-induced hypertension. The *DICER1* rs1078 polymorphism is associated with GH and the *RAN* rs3803012 polymorphism is associated with PE.

1. Introduction

Pregnancy-induced hypertension (PIH), which includes gestational hypertension (GH) and preeclampsia (PE), complicates 5–10% of pregnancies and is a major cause of maternal, fetal, and neonatal morbidity and mortality [1,2]. Furthermore, PIH could increase the risk of long-term cardiovascular diseases of both mothers and their offspring [3,4]. Although significant efforts have been made, little is known about the etiology of PIH [2].

MicroRNAs (miRNAs) are a subclass of ~22 nucleotide single-strand noncoding RNA molecules that function principally by disrupting target messenger RNA (mRNA) expression and influencing cellular functions at the pathway level [5]. Dysregulated miRNAs have been involved in the pathogenesis of many human diseases, such as cancer, cardiovascular diseases, and psychiatric and neurological diseases [6–8]. Increasing evidence suggests the involvement of miRNAs in PIH development: compared with normal pregnancy, PE patients have altered placental miRNA expression [9]; circulating serum/plasma

Abbreviations: miRNA, microRNA; PIH, pregnancy induced hypertension; GH, gestational hypertension; PE, preeclampsia; SNPs, single nucleotide polymorphisms; GDM, gestational diabetes mellitus; FDR, false-positive discovery rate; GMDR, generalized multifactor dimensionality reduction; VEP, variant Effect Predictor; TFBS, transcriptional factor binding sites

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miRNAs expression in early gestation might predict later onset of PE and GH [10].

The biosynthesis of human miRNAs involves a multiple-step process that starts in the nucleus of the cell, continues with transcription, and then continues through the cytoplasm, where the mature miRNA molecule exerts its main function [11]. Once one or more of the multiple steps involved in miRNAs biosynthesis is disturbed, it causes widespread downstream perturbations [12]. For example, single nucleotide polymorphisms (SNPs) within the miRNA machinery genes are found to result in altered miRNA expression [13] that has been associated with cancer [14–17], stroke [18], neurological diseases [19], idiopathic primary ovarian insufficiency [20] and idiopathic recurrent pregnancy loss [21].

Given that PIH is associated with deregulation of miRNAs [9,10], and that miRNA expression levels could be affected by miRNA machinery gene polymorphisms [13], we therefore hypothesized that PIH might be associated with miRNA machinery gene polymorphisms. To the best of our knowledge, only two studies conducted in Iran have tested the association between maternal *DORSHA*, *DICER1* polymorphisms and PE [22,23]. The differences in the genetic predisposition for GH and PE have been recognized [24]. However, in the Iranian studies, the association between *DORSHA* or *DICER1* polymorphisms and GH was not discussed. Moreover, different populations have different genetic predispositions for PIH [25]. The generalizability of Iranian population results to other populations needs further investigation. Except for *DICER1* and *DORSHA,* other miRNA machinery genes, such as *RAN* and *XPO5* have also been associated with human reproductive diseases [17,20–21]. We therefore have genotyped 9 SNPs, including *DICER1* (rs3742330, rs1057035 and rs13078), *DROSHA* (rs17409893 and rs642321), *RAN* (rs3803012 and rs14035), and *XPO5* (rs1106841 and rs2257082), in a case-control study to evaluate the impact of these genes polymorphisms on the risk of GH and PE among Han Chinese women.

2. Methods

2.1. Study population

We enrolled 130 patients diagnosed with PE, 67 with GH, and 300 healthy controls from the Liuyang Municipal Hospital of Maternal and Child Health (LYMHMCH), and we enrolled another 13 patients diagnosed with PE, 12 with GH, and 30 healthy controls from the Third Xiangya Hospital of Central South University (TXYHCSU). The enrollment criteria for the cases and controls were the same as those used at two hospitals that were described in our previously published study [26]. Briefly, the enrollment criteria for cases were pregnant women with a clinical diagnosis of GH or PE patients without chronic diabetes mellitus, renal disease, or chronic cardiovascular diseases (including chronic hypertension), and for controls were normotensive pregnant women without chronic diseases who delivered at the same hospital during the same period.

2.2. Data collection and diagnosis criteria

The study protocol was reviewed and approved by the Ethical and Confidentiality Committee of Central South University (Reference XYGW-2016-01, approved on 3rd Mar 2016) and by the institutional review boards from LYMHMCH. All participants were informed about the case-control study upon their arrival at the hospital for delivery. After obtaining written consent, 5 ml peripheral blood samples were aseptically collected and stored in a 4℃ fridge in EDTA anticoagulant tubes and were processed for DNA extraction within 24 h. Meanwhile, a self-administered questionnaire was distributed to participants for collecting demographic, reproductive and medical history data. Information on maternal complications was abstracted from the medical records. PIH cases included both GH and PE conditions. GH was diagnosed as hypertension (measured twice 6 h apart, ≥140/90 mmHg) that manifested after 20 weeks of gestation without proteinuria. PE was diagnosed as hypertension concurrent with proteinuria (≥1 + on dipstick in two urine samples) after 20 weeks of gestation, or without proteinuria but showed symptoms of thrombocytopenia, impaired liver function, new development of renal insufficiency, pulmonary edema or new onset of cerebral or visual disturbance.

2.3. SNP selection and genotyping

Through the 1000 Genomes project (http://www.internationalgenome.org/) and dbSNP (https://www.ncbi.nlm.nih.gov/snp/), this study identified 9 potential functional SNPs, including three *DICER1* SNPs (rs3742330, rs1057035 and rs13078), two *DROSHA* SNPs (rs17409893 and rs642321), two *RAN* SNPs (rs3803012 and rs14035), and two *XPO5* SNPs (rs1106841 and rs2257082). All selected SNPs have been reported with a minor allele frequency (MAF) > 0.05 in the Han Chinese population and are located in a potentially functional area, including exons, UTRs and promoters (within 2 kb of the genes) (Table S1).

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.

2.4. Statistical analysis

Chi-squared tests and ANOVA tests were used to compare the distribution of maternal characteristics between PE, GH and controls for categorical variables and continuous variables, respectively. When the overall chi-square test or ANOVA test was significant, the partition of the chi-square method ($\alpha' = \alpha/(3*(3–1)/2) + 1) = 0.0125$) or the Student-Newman-Keuls test, respectively, was adopted for further comparison between each of the two groups. Allele frequencies were calculated to identify deviations from Hardy–Weinberg equilibrium (HWE) in the controls. Unconditional logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (CIs) for the associations between different genotypes, different allele combination frequencies and risk of GH and PE, respectively. Potential confounding variables included maternal age (< 25, 25–34, and ≥ 35 years), gestational age at delivery (< 37 and ≥ 37 weeks), nulliparous (yes or no), GDM (yes or no), active and/or passive smoking during pregnancy (yes or no), previous PIH (yes or no) and fetal gender (male or female). For further exploring the gene-gene interactions, the generalized multifactor dimensionality reduction (GMDR) method was first adopted among the SNP loci with GMDR 0.9 software. When a statistically significant interaction model was found, further analysis on the association between the allele combination of the selected models and risk of PIH/GH/PE was evaluated. The false-positive discovery rate (FDR) correction was adopted for multiple comparisons. Statistical significance was assessed at the 5% level (two-tail test). All analyses were performed using SAS software, version 9.2 (SAS Institute, Inc., Cary, NC). To better evaluate the impact of these selected SNPs on the expression of the proteins, Ensembl's Variant Effect Predictor (VEP) tool was utilized (http://grch37.ensembl.org/info/docs/tools/vep/index.html).

3. Results

In total, 143 patients diagnosed with PE, 79 with GH, and 330 healthy controls were recruited and analyzed. Compared to controls, GH and PE cases were more likely to be older, to give birth at an earlier gestational age and to have PIH history ($p < 0.05$) (Table 1). GH cases were more likely to suffer from GDM than the other two groups ($p < 0.0125$). There were no significant differences in the distribution of parity, exposure to active and/or passive smoking and fetal gender between GH/PE cases and controls (Table 1).

Table 1
Distributions of characteristics between selected PIH patients and Controls.

Characteristics	Control (N = 330)	GH (N = 79)	PE (n = 143)	*p*
Maternal age, years (mean ± SD)	27.98 ± 3.82[a]	29.26 ± 5.41[a]	29.36 ± 5.73[a]	0.004
Gestational age at delivery, weeks (mean ± SD)	39.35 ± 2.97[b]	38.56 ± 1.64[b]	37.20 ± 3.29[b]	< 0.001
Nulliparous (n, %)				
Yes	152 (46.06)	46 (58.23)	68 (47.55)	0.149
No	178 (53.94)	33 (41.77)	75 (52.45)	
Active and/or passive smoking				
Yes	102 (30.90)	31 (39.24)	46 (32.16)	0.363
No	228 (69.09)	48 (60.75)	97 (67.83)	
Fetal gender				
Male	167 (50.61)	33 (41.77)	65 (45.45)	0.280
Female	163 (49.39)	46 (58.23)	78 (54.55)	
Previous PIH				
Yes	1 (0.30) [c]	1 (1.26) [c]	11 (7.69) [c]	< 0.001
No	229 (99.70)	78 (98.73)	132 (92.31)	
GDM				
Yes	17 (5.15) [d]	18 (22.78) [d]	13 (9.09) [d]	< 0.001
No	313 (94.85)	61 (77.22)	130 (90.91)	

a. Student-Newman-Keuls Test, control compared with GH, $p < 0.05$; control compared with PE, $p < 0.05$; GH compared with PE, $p > 0.05$;
b. Student-Newman-Keuls Test, control compared with GH, $p < 0.05$; control compared with PE, $p < 0.05$; GH compared with PE, $p < 0.05$;
c. Control compared with GH, $p > 0.0125$, control compared with PE, $p < 0.0125$, GH compared with PE, $p > 0.0125$;
d. Control compared with GH, $p < 0.0125$, control compared with PE, $p > 0.0125$, GH compared with PE, $p < 0.0125$;
Abbreviation: PIH, pregnancy induced hypertension; GH, gestational hypertension; PE, preeclampsia; GDM, Gestational Diabetes Mellitus.

The genotyping completion rates were > 98% for all SNPs. All of the genes that were analyzed have showed polymorphisms and were observed with HWE in both of the case and control groups. The results from VEP showed that rs1106841 and rs2257082 had predicted 'low' effects and the rest of the SNPs were categorized as having a 'modifier' impact (Table S1).

The distributions of the genotypes of the 9 selected SNPs and their associations with risk of GH and PE are presented in Table 2. After adjusting for maternal age, gestational age at delivery, parity, history of PIH, gestational diabetes, smoking during pregnancy, and fetal gender, the *DICER1* rs13078 TA genotype was still strongly associated with increased risk of GH (the adjusted OR = 3.17 (95% CI: 1.55, 6.48), $p = 0.001$), and the *RAN* rs3803012 AG genotype was associated with increased risk of PE (the adjusted OR = 2.15 (95% CI: 1.26, 3.66), $p = 0.005$). Those associations were retained after FDR correction ($p < 0.05$). Compared with the frequencies observed in the controls, the frequencies of the *XPO5* rs1106841AC genotype were higher in the GH group. However, this associations became insignificant after FDR correction ($p = 0.07$).

The associations between possible haplotypes and the risk of GH and PE were estimated and are listed in Table 3. The *RAN* rs14035/ rs3803012 haplotype C-G was significantly associated with increased risk of PE (OR = 2.08 (95% CI: 1.19, 3.62), $p = 0.008$); however, the haplotype C-A was associated with lower risk of PE (OR = 0.68 (95% CI: 0.50, 0.93), $p = 0.016$). The *DICER1* rs1057035/ rs13078/ rs3742330 haplotype T-A-A and the *DORSHA* rs17409893/ rs642321 haplotype A-T were significantly associated with increased risk of GH, and the adjusted ORs were 2.75 (95% CI:1.40, 5.38) ($p = 0.002$) and 1.60 (95% CI:1.12, 2.29)($p = 0.008$), respectively (Table 3). Those associations were retained after FDR correction ($p < 0.05$).

To explore whether gene-gene interactions have synergistic effects on the risk of GH or PE, GMDR analysis was conducted. However, none of the interaction models won all 10 rounds of cross validation (Table S2). Therefore, no further allele combination analysis was conducted.

4. Discussion

As far as we know, our study is the first epidemiological study evaluating the effects of 9 SNPs in *DICER1*, *DORSHA, RAN* and *XPO5* and the risk of GH and PE in the Han Chinese population. Our results showed that the *DICER1* rs1078 genotype TA is associated with increased risk of GH and that the *RAN* rs3803012 AG genotype is associated with increased risk of PE in the Han Chinese population. *DICER1* and *DORSHA* haplotypes are significantly associated with risk of GH. However, *RAN* haplotypes are associated with risk of PE.

As an RNase III superfamily member, *DORSHA* initiates miRNA processing by converting pri-miRNA into pre-miRNA [12]. Dorsha is involved in angiogenesis and coagulation mechanisms [26]. A higher frequency of the *DORSHA* rs10719 TC genotype was found among women with PE in Iran, suggesting the possible involvement of this *DORSHA* polymorphism in PE development [22]. The *DORSHA* rs642321 polymorphism in the 3′-UTR region and the rs17409893 polymorphism in the promoter region were associated with male infertility among Han Chinese [27]. In the current study, we have first evaluated the possible effects of those two SNPs on GH and PE susceptibility. The results suggest that neither of those two SNPs is directly associated with GH or PE. However, *DORSHA* rs17409893/ rs642321 haplotype A-T is associated with increased risk of GH. Considering no previous study has elucidated the functions of those SNPs in miRNA biogenesis, AliBaba software was used to analyze the putative transcriptional factor binding sites (TFBS) [28]. We found that genetic variants in rs642321 and rs17409893 might not alter the binding sites (data not shown). More case-control studies may be needed to validate our findings.

Dicer is an enzyme responsible for the cleavage of miRNA precursors, and is known as a critical regulator of the biogenesis of miRNA [12]. Emerging evidence shows that polymorphisms in *DICER* may alter its biologic functions and play important roles in the development of various diseases, including stroke [18], idiopathic primary ovarian insufficiency [19] and idiopathic recurrent pregnancy loss [20]. A recent study conducted in an Iranian population found that the *DICER1* rs3742330 AG genotype in placenta is significantly associated with increased risk of maternal severe PE (OR = 3.0 (95% CI: 1.3, 6.4)) [23]. *DICER1* SNP rs3742330, rs13078 and rs3742330 are all located in the 3′-UTR, which may affect mRNA stability and subsequent expression through changing the binding capacity of regulatory miRNAs [29]. Knockdown of Dicer in human placenta could cause a global reduction in miRNA and significantly enhance cytotrophoblast proliferation [30] which is a major pathogenic even in preeclampsia. However, maternal *DICER1* polymorphism is not statistically related to Dicer1 mRNA dysregulation in placenta [23]. Consistent with findings in an Iranian population [23], an insignificant association between maternal *DICER1*

Table 2
Associations between the genotypes of miRNA machinery genes and PIH.

Genotype	Controls	GH				PE			
	N (%)	N (%)	OR^{a}_{adj} (95%CI)	*p*	*p* [b]	N (%)	OR^{a}_{adj} (95%CI)	*p*	*p* [b]
DICER1 rs1057035									
TT	244(74.16)	52(65.82)	Reference			103(72.03)	Reference		
TC	79(24.01)	24(30.38)	1.40 (0.82, 2.46)	0.208	0.374	36(25.17)	1.06 (0.68, 1.70)	0.747	0.911
CC	6(1.82)	3(3.80)	2.40 (0.57, 10.07)	0.226	0.678	4(2.80)	1.55 (0.43, 5.70)	0.488	0.698
DICER1 rs13078									
TT	307(93.03)	64(81.01)	Reference			132(92.96)	Reference		
TA	22(6.67)	15(18.99)	3.17 (1.55, 6.48)	0.001	0.009	9(6.34)	0.94 (0.42, 2.13)	0.911	0.911
AA	1(0.30)	0(0.00)	–			1(0.70)	2.30 (0.14, 37.44)	0.552	0.737
DICER1 rs3742330									
AA	128(39.02)	39(49.37)	Reference			51(35.66)	Reference		
AG	150(45.73)	28(35.44)	0.60 (0.34, 1.01)	0.063	0.142	68(47.55)	1.15 (0.72, 1.73)	0.598	0.897
GG	50(15.24)	12(15.19)	0.75 (0.37, 1.59)	0.476	0.707	24(16.78)	1.20 (0.67, 2.17)	0.529	0.698
DROSHA rs17409893									
AA	209(63.33)	55(69.62)	Reference			91(63.64)	Reference		
AG	107(32.42)	20(25.32)	0.70 (0.41, 1.26)	0.257	0.386	49(34.27)	1.06 (0.69, 1.59)	0.819	0.911
GG	14(4.24)	4(5.06)	1.24 (0.38, 4.03)	0.707	0.707	3(2.10)	0.48 (0.13, 1.73)	0.265	0.698
DROSHA rs642321									
CC	88(26.67)	17(21.52)	Reference			40(27.97)	Reference		
CT	178(53.94)	39(49.37)	1.11 (0.60, 2.10)	0.706	0.794	69(48.25)	0.83 (0.53, 1.35)	0.500	0.897
TT	64(19.39)	23(29.11)	1.80 (0.89, 3.70)	0.099	0.594	34(23.78)	1.16 (0.66, 2.04)	0.582	0.698
RAN rs14035									
CC	225(68.18)	54(68.35)	Reference			89(62.68)	Reference		
CT	94(28.48)	23(29.11)	1.01 (0.59, 1.76)	0.942	0.942	48(33.80)	1.27 (0.84, 1.97)	0.238	0.897
TT	11(3.33)	2(2.53)	0.66 (0.14, 3.19)	0.624	0.707	5(3.52)	1.12 (0.37, 3.32)	0.840	0.840
RAN rs3803012									
AA	299(91.16)	70(88.61)	Reference			118(82.52)	Reference		
AG	29(8.84)	9(11.39)	1.32 (0.60, 2.96)	0.472	0.607	25(17.48)	2.15 (1.26, 3.66)	0.005	0.045
GG	0(0.00)	0(0.00)	–			0(0.00)	–		
XPO5 rs1106841									
AA	299(90.61)	64(81.01)	Reference			132(92.31)	Reference		
AC	31(9.39)	15(18.99)	2.31 (1.17, 4.57)	0.015	0.067	11(7.69)	0.81 (0.39, 1.64)	0.552	0.897
CC	0(0.00)	0(0.00)	–			0(0.00)	–		
XPO5 rs2257082									
TT	137(41.52)	24(30.38)	Reference			68(47.55)	Reference		
TC	156(47.27)	46(58.23)	1.75 (1.02, 3.06)	0.042	0.126	64(44.76)	0.80 (0.54, 1.24)	0.364	0.897
CC	37(11.21)	9(11.39)	1.41 (0.60, 3.37)	0.419	0.707	11(7.69)	0.60 (0.28, 1.22)	0.156	0.698

a Adjustment covariates are maternal age, gestational age at delivery, nulliparous, chronic disease before pregnancy, previous PIH, GDM and fetal gender,;
b FDR adjusted *p* value;
Abbreviation: PIH, pregnancy induced hypertension; GH, gestational hypertension; PE, preeclampsia; GDM, Gestational Diabetes Mellitus

Table 3
Associations between haplotypes of miRNA machinery genes and PIH [a].

Haplotype	Control	GH				PE			
	freq	freq	OR^{b}_{adj}(95%CI)	*p*	*p*[c]	freq	OR^{b}_{adj}(95%CI)	*p*	*p*[c]
DICER1 rs1057035/ rs13078/ rs3742330									
T-T-A	0.441	0.386	0.80 (0.55, 1.13)	0.203	0.214	0.405	0.85 (0.64, 1.14)	0.293	0.828
T-T-G	0.382	0.329	0.79 (0.43, 1.14)	0.214	0.214	0.405	1.10 (0.82, 1.46)	0.513	0.828
T-A-A	0.036	0.095	2.75 (1.40, 5.38)	0.002	0.008	0.039	1.05 (0.52, 2.19)	0.879	0.879
C-T-A	0.139	0.190	1.45 (0.92, 2.28)	0.108	0.214	0.151	1.10 (0.73, 1.63)	0.621	0.828
DORSHA rs17409893/ rs642321									
A-C	0.492	0.411	0.72 (0.51, 1.02)	0.066	0.132	0.479	0.94 (0.71, 1.25)	0.704	0.999
A-T	0.303	0.411	1.60 (1.12, 2.29)	0.008	0.032	0.328	1.12 (0.83, 1.51)	0.433	0.999
G-C	0.043	0.050	1.16 (0.52, 2.58)	0.287	0.716	0.041	0.95 (0.47, 1.89)	0.890	0.999
G-T	0.160	0.126	0.75 (0.45, 1.26)	0.716	0.382	0.150	0.92 (0.62, 1.35)	0.690	0.999
RAN rs14035/ rs3803012									
C-A	0.780	0.772	0.97 (0.64, 1.48)	0.922	0.922	0.707	0.68 (0.50, 0.93)	0.016	0.024
T-A	0.175	0.170	0.97 (0.61, 1.54)	0.920	0.922	0.204	1.20 (0.83, 1.71)	0.296	0.296
C-G	0.044	0.056	1.31 (0.60, 2.83)	0.484	0.922	0.088	2.08 (1.19, 3.62)	0.008	0.024
XPO5 rs1106841/ rs2257082									
A-T	0.651	0.594	0.78 (0.55, 1.12)	0.183	0.274	0.699	1.24 (0.92, 1.67)	0.152	0.361
A-C	0.301	0.310	1.04 (0.71, 1.51)	0.832	0.832	0.262	0.82 (0.60, 1.12)	0.221	0.361
C-C	0.046	0.094	2.12 (1.12, 4.04)	0.018	0.054	0.038	0.81 (0.40, 1.63)	0.559	0.610

a. All those frequency < 0.03 were ignored in analysis;
b. Adjustment covariates are maternal age, gestational age at delivery, nulliparous, active and/or passive smoking during pregnancy, previous PIH, GDM, and fetal gender;
c. FDR adjusted *p* value.
Abbreviation: PIH, pregnancy induced hypertension, GH, gestational hypertension, PE, preeclampsia.

polymorphism and PE was found in this Han Chinese population.

Although GH and PE have many shared risk factors, contemporary evidence suggests that GH and PE are distinct entities with different mechanisms [24]. Our results found that *DICER1* rs13078 genotype TA and rs3742330/ rs13078 /rs3742330 haplotype T-A-A were only associated with GH, but were not associated with PE. *DICER1* rs13078 polymorphisms could affect endothelial miRNA expression [31] which is clearly associated with the vascular endothelial dysfunction and development of chronic hypertension. The mechanism for GH includes the manifestation of underlying predisposition toward chronic hypertension during pregnancy. We speculated that this may be the reason for the observed different associations.

In the miRNA processing system, the XPO5/RAN-GTP complex mediates the nuclear transport of pre-miRNAs [12]. *XPO5* rs1106841 and rs2257082 are both located in the extron area and are associated with the development of various cancers [13,14] and idiopathic primary ovarian insufficiency [20]. However, none of their polymorphisms were significantly associated with GH/PE in our study. According to VEP, polymorphisms in rs2257082 and rs1106841both had predicted 'low' effects, meaning that their polymorphisms are unlikely to change protein behavior.

The Ran protein regulates a wide variety of cellular processes [12]. An abundance of Ran protein in maternal plasma at 8–28 weeks of gestation was found in PE cases [32], which support its role in PE development. The rs14035, rs3803012 polymorphisms in the RAN 3′-UTR were associated with increased risk of various kinds of cancers including colorectal, hepatocellular and cervical cancers [14–16]. Our study also observed an increased risk of PE among those with the *RAN* rs3803012 AG genotype and the *RAN* rs14035/ rs3803012 haplotype C-G. Putative TFBS analysis showed that the G allele of rs3803012 might lead to loss of the binding sites for TATA-binding protein (TBP) and CCAAT-enhancer-binding proteins (C/EBP) beta (Figure S1). In such cases, the *RAN* gene is no longer regulated by the original transcriptional factor which might finally results in differences in gene expression, phenotypes and susceptibility to environmental exposure [33]. However, the specific molecular mechanism needs further experimental validation.

Our study has several strengths in comparison with previous studies in the field. The data contained detailed information on maternal demographic and medical information allowing adjustment for important potential confounding factors. Diagnosis of GH/PE in our study was based on medical records, not self-report, which minimized potential disease misclassifications. However, the limitation of our study should not be ignored when interpreting the study findings. Our case-control study only explores the association between SNPs in miRNA machinery genes and GH/PE development, not the causal relationship. The functional study of those SNPs to elucidate the potential mechanism for how polymorphisms in miRNA machinery genes could affect PIH development has not been conducted in the present study. Moreover, our study was conducted among Chinese with a relatively small sample size. The replication of our finding in an independent population is needed.

5. Conclusion

Our studies confirmed that genetic variants in microRNA machinery genes might participate in the pathologic process of pregnancy induced hypertension. The *DICER1* rs1078 T > A polymorphism is associated with gestational hypertension susceptibility and the *RAN* rs3803012 A > G polymorphism is associated with preeclampsia susceptibility. Moreover, the *DICER1* and *DORSHA* haplotypes are significantly associated with the risk of GH, and *RAN* haplotypes are associated with the risk of PE. Determining the mechanism on how those SNPs modify the susceptibility to pregnancy-induced hypertension requires further research.

6. Author's contribution

Jun Lei and Hongzhuan Tan have designed the study and directed its implementation; Xin Huang conducted the literature review, statistical analysis, and drafted the manuscript; Dapeng Wang did the data interpretation and experimental work including Genomic DNA extraction and genotyping; Xun Li participated in the data clean and statistical analysis; Yuhui An designed and supervised the field activities (follow-up and data collection) and data interpretation. All authors read and approved the final manuscript.

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Competing Interests Declaration

The authors declare that they have no conflicting interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2019.04.005.

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