Functional polymorphisms in the MED13L and SLC4A7 genes are associated with pregnancy-induced hypertension

Ying Zhou

Central South University Xiangya School of Medicine

Xun Li

Hunan Children's Hospital

Shimin Hu

Central South University Xiangya School of Public Health

Shujin Zhou

Central South University Xiangya School of Medicine

Hongzhuan Tan (✉ tanhz99@qq.com)

Central South University Xiangya School of Public Health <https://orcid.org/0000-0001-9764-1813>

Research article

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Abstract

Background: Pregnancy-induced hypertension (PIH), including gestational hypertension (GH) and preeclampsia (PE), might share pathophysiologic mechanisms and genetic risk factors with hypertension. This study aimed to investigate whether genetic risk factors for hypertension also been associated with PIH. **Methods:** This is a case control candidate gene association study. A total of 84 cases with GH, 108 cases with PE, and 192 age-matched (± 1 year) controls were recruited from the Liuyang Municipal Hospital of Maternal and Child Health, Hunan Province of China. Three candidate single nucleotide polymorphisms (SNPs) which have been found to be associated with hypertension or blood pressure (BP) were chosen. The associations between SNPs and GH and PE were analyzed by multiple logistic regression models after adjusting for fetal sex and primipara. **Results:** GG genotype at mediator complex subunit 13-like (*MED13L*) rs11067763 was a protective factor for developing GH (GG vs. AA + AG, OR = 0.376, 95%CI = 0.158-0.892) and PE (GG vs. AA + AG, OR = 0.489, 95%CI = 0.245-0.979) in recessive model. AA genotype at solute carrier family 4 member 7 (*SLC4A7*) rs820430 was a risk factor for developing GH (AA vs. GG + AG, OR = 3.562, 95%CI = 1.343-9.447) and PE (AA vs. GG + AG, OR = 3.351, 95%CI = 1.352-8.303) in recessive model. But no significant association was observed between cytochrome P450 family 21 subfamily A member 2 (*CYP21A2*) rs2021783 and the GH/PE groups. **Conclusions:** PIH and hypertension are likely to share genetic risk factors. The associations both between variants of *MED13L* rs11067763 and GH/PE and between variants of *SLC4A7* rs820430 and GH/PE were significant.

Background

Pregnancy-induced hypertension (PIH, including gestational hypertension [GH] and preeclampsia [PE]) is a pregnancy-specific complication affecting 5-10% of all pregnant women in the world [1]. Both GH and PE are characterized by hypertension first appearing after 20 weeks of gestation, while PE is also characterized by new onset proteinuria [2]. PIH is a leading cause of maternal and fetal morbidity and mortality. Without knowing the exact etiology of PIH, delivery of the baby is the only effective way when the lives of the pregnant women are threatened [3, 4].

Since familial association was observed for PIH, inheritance could have played a major function in the pathology of PIH. A twin study suggested the heritability of GH and PE was 24% and 54%, respectively [5]. Besides, it has been reported that maternal genetic factors contribute to PIH susceptibility[6]. Epidemiological studies also have shown that a family history of chronic hypertension is more common in PE groups compared with controls, and PIH is associated with an increased long-term risk of maternal hypertension[7]. Studies on pathophysiologic mechanisms have demonstrated endothelial dysfunction, inflammation, and vascular remodeling to be crucial to the development of chronic hypertension, as well as PIH [8, 9]. In line with these findings, it has been suggested that PIH and hypertension might share pathophysiological factors. And the genetic risks predisposing to hypertension and PIH may thus be partially overlapping [8, 10, 11].

Genome-wide association studies (GWAS) have been conducted to search for hypertension-susceptibility genes. Recently, Chinese researchers identified that mediator complex subunit 13-like (*MED13L*) rs11067763, solute carrier family 4 member 7 (*SLC4A7*) rs820430, and cytochrome P450 family 21 subfamily A member 2 (*CYP21A2*) rs2021783 were possibly genetic variants associated with blood pressure (BP) and/or hypertension[12]. Further studies found that hypertension risk SNP rs820430 functioned as an enhancer of *SLC4A7* [13]. Another GWAS carried out in the USA found associations between *CYP17A1/CYP1A2* and BP [14]. Considering the overlapping of genetic risks of hypertension and PIH, it is valuable to investigate the relationship between these three SNPs and PIH.

The present study aims to detect the association between three SNPs, which were previously reported having association with BP or hypertension, and PIH in a Southern Han Chinese population.

Materials And Methods

Study population

Statistical analyses were conducted using IBM SPSS 19.0 software (IBM SPSS, Inc., Chicago, IL). A total of 108 patients with PE, 84 with GH, and 192 controls were recruited from the Liuyang Municipal Hospital of Maternal and Child Health, Hunan Province of China. The inclusion criteria for the case group were clinical diagnosis of GH or PE without diabetes mellitus, renal disease, cerebrovascular disease (CVDs), or other diseases that are already known as risk factors for PIH, and HELLP syndrome was excluded. The inclusion criteria for the control group were age-matched (± 1 year) normotensive pregnant women who delivered at the same hospital during the same period and with the absence of aforementioned diseases.

Ethics statement

This study conducted in accordance with the 1975 Declaration of Helsinki and was reviewed and approved by the Committee for Human Research Protections of Xiang-Ya School of Medicine. Informed consent was obtained from each individual included in the study, after which blood samples and medical records were collected.

Diagnostic criteria

PE was defined according to the International Society for Study of Hypertension in Pregnancy as having de novo hypertension (systolic blood pressure[SBP] ≥ 140 mmHg or diastolic blood pressure[DBP] ≥ 90 mmHg after the 20th week of gestation) and accompanying proteinuria (urinary protein dip sticks $\geq 2+$ or ≥ 300 mg in a 24-h urine sample). GH was similarly defined as de novo hypertension without proteinuria [15, 16].

SNP selection and genotyping

Three candidate SNPs were selected, including *MED13L* rs11067763, *SLC4A7* rs820430, and *CYP21A2* rs2021783. Genomic DNA was extracted from whole blood using the TIANamp Blood DNA Kit (DP318-03, TIANGEN, Beijing). Genotyping of the three SNPs was performed using matrix-assisted laser desorption/ionization-time of flight(MALDI-TOF) mass spectrometry, according to the manufacturer's instructions[17].

Statistical analysis

Statistical analyses were conducted using IBM SPSS 19.0 software (IBM SPSS, Inc., Chicago, IL). Mean $\pm$ standard deviation (SD), or median and interquartile were used to describe the continuous variables with normal or skewed distribution, respectively. Frequency was used to describe categorical variables. To compare demographic and clinical data, the t-test or Wilcoxon rank sum test was used for continuous variables, and the Chi-square test or Fisher's exact test was used for categorical variables. The Bonferroni correction was applied for multiple comparisons ($\alpha = 0.05/3 = 0.0167$). The SNP detection rate was calculated as the number of sites that were successfully genotyped for all of the samples divided by the number of genotyped sites for all samples. The Hardy-Weinberg test was conducted in control groups using the Chi-square goodness-of-fit test or the Fisher's exact test ($\alpha = 0.01$). The Chi-square test or Fisher's exact test was used to test the allele/genotype distribution between the case

and control groups. Univariate logistic regression was used to estimate the crude odds ratio (OR) and its corresponding 95% confidence interval (95% CI) for different genetic models, including dominant model and recessive model. Through multiple logistic regression, the adjusted OR and 95% CI were also calculated after adjusting for fetal sex and primipara[18, 19].

Results

Demographic and clinical features

In total, 84 GH cases, 108 PE cases, along with 192 controls were analyzed. The main demographic and clinical features of three groups are summarized in Table 1. No significant between-group differences were detected for fetal sex, primipara, and new born weight ($P > 0.0167$). Meanwhile, serum uric acid, in-hospital SBP, and in-hospital DBP with GH and PE were significantly higher than those in normotensive women. Moreover, in-hospital SBP of PE women were significantly higher than those in GH women, whereas there were no significant differences between the GH and PE groups as to other characteristics. Interestingly, creatinine of women with PE were significantly higher than those in GH and control groups, and gestational age of PE groups were significantly lower than those in GH and control groups, while no significant differences were found between GH and control groups on these two factors.

SNPs and PIH

The SNP detection rate was 98%. The allele frequencies of control groups showed no deviation from Hardy-Weinberg equilibrium, shown the representativeness of our control group. The distributions of genotypes and alleles among the three groups are shown in Table 2. The results of the Chi-square test showed that the distribution of *MED13L* rs11067763 genotype was significantly different between the GH and control groups ($P = 0.007$), and there was significant difference in the *SLC4A7* rs820430 allele between the GH and control groups ($P = 0.043$), but no significant differences in these two SNPs genotype/allele were observed between the PE and control groups. Furthermore, no significant differences in *CYP21A2* rs2021783 genotype/allele were found between the case and control groups.

The univariate logistic regression results are shown in Table 3. Two SNPs showed nominal association with GH or PE. Specifically, *MED13L* rs11067763 was significantly associated with both GH (GG vs. AA + AG, $P = 0.009$) and PE (GG vs. AA + AG, $P = 0.034$) in recessive model. Also, *SLC4A7* rs820430 was significantly associated with both GH (AA vs. GG, $P = 0.021$; AA vs. GG + AG, $P = 0.020$) and PE (AA vs. GG + AG, $P = 0.026$). However, no significant association was observed between *CYP21A2* rs2021783 and the GH/PE groups.

After adjusted for fetal sex and primipara by logistic regression, most of the results remained the same as the univariate logistic regression results (Table 3). *MED13L* rs11067763 was significantly associated with both GH and PE, being a carrier of the GG genotype was associated with a decreased susceptibility of developing GH/PE (GG vs. AA + AG, $P = 0.027$; and GG vs. AA + AG, $P = 0.043$, respectively); *SLC4A7* rs820430 was significantly associated with both GH and PE, being a carrier of the AA genotype was associated with an increased susceptibility of developing GH/PE (AA vs. GG, $P = 0.007$, AA vs. GG + AG, $P = 0.011$; and AA vs. GG, $P = 0.026$, AA vs. GG + AG, $P = 0.009$, respectively); being a carrier of the *SLC4A7* rs820430 A allele was associated with an increased susceptibility of developing GH (A vs. G, $P = 0.033$). However, still no significant association was detected between *CYP21A2* rs2021783 and the GH/PE groups in multiple logistic regression analysis.

Discussion

The typical manifestation of PIH is BP elevation. Women with a history of PIH are more susceptible to chronic hypertension and women with chronic hypertension are at increased risk for PE. Luscher demonstrated that endothelial cell injury in PIH women could profoundly affect cardiovascular function, and patients were likely to become hypertensive, hyperlipidemic, and eventually develop atherosclerosis with aging[7]. Ellen et al. reported that women with chronic hypertension had an increased rate of PE (17-25%, vs. 3-5% in the general population)[20]. Women with chronic arterial hypertension were also at increased risk for superimposed PE [21]. Except for similar metabolic bases, PIH and hypertension share some genetic risk factors. Our team had found significant associations between angiotensinogen (*AGT*) rs3789678 and GH and between angiotensin II receptor type 1 (*AGTR1*) rs275645 and PE after adjusting for fetal sex, maternal age, and gestational diabetes mellitus (GDM) [11]. So validate the association between hypertension candidate genes and PIH is a reasonable and effective way to find genetic risk factors for PIH.

In this case-control study, significant differences were found between *MED13L* rs11067763 and GH/PE, and *SLC4A7* rs820430 associated with the onset and development of GH/PE. In detail, carrying the GG genotype at rs11067763 was a protective factor for developing GH/PE. As for rs820430, carrying the AA genotype was a risk factor for developing GH/PE. But no significant association between *CYP21A2* rs2021783 polymorphisms and GH/PE was found. Importantly, our study reveals for the first time that the *MED13L* rs11067763 and *SLC4A7* rs820430 were associated with GH/PE risk among Chinese Han women.

MED13L is a paralog of the closely related protein named Mediator complex subunit 13 (*MED13*), which is believed to have arisen from gene duplication events[22]. It is responsible for bridging the RNA pol II enzyme and transcription factors to initiate transcription of protein-coding genes and non-coding RNA genes [23]. Through retinoblastoma (Rb)/E2F pathways, it promotes proliferation of vascular smooth muscle cells which are important in the development of hypertension [24]. Besides, several researches have shown an indirect association between *MED13L* and inflammation through Wnt pathway which plays a regulatory role in immune system[25]. Moreover, *MED13L* regulates the expression level of activating transcription factor 4 (ATF4) which regulates the secretion of pro-inflammatory cytokines such as interleukin (IL)-6 and IL-8[26, 27], and these two cytokines have been considered influencing the onset of GH and PE. As Adela et al. reported, the serum level of IL-6 decreased in GH subjects [28]. Besides, Krasnyi found that increased endothelial IL-8 expression represented endothelial dysfunctions, which are significant pathophysiologic features of PE [29]. In our study, we found genetic variants of *MED13L* rs11067763 associated with development of GH/PE in Chinese Han women. GG genotype at rs11067763 has shown to be a protective factor for GH/PE. Thus, more studies are needed to validate the association in larger population, and researches on mechanisms are necessary to uncover the molecular pathways of the association.

SLC4A7 gene is on chromosome 3p24.1, it belongs to the SLC4 family [30]. *SLC4A7* encodes the electroneutral $\text{Na}^+/\text{HCO}_3^-$ co-transporter NBCn1. Several researchers have demonstrated that NBCn1 has a role in controlling intracellular pH (pHi) in vascular smooth muscle cells (VSMCs), and pHi is a determinant of VSMCs contractility and endothelial function, both of which effect BP. Furthermore, through elevated steady-state pHi and accelerated recovery from intracellular acidosis, they also found genetic variants of *SLC4A7* rs13082711 associated with increased *SLC4A7* expression level and NBCn1 function.[31-34]. A large scale GWAS also detected association between polymorphisms of *SLC4A7* rs13082711 and SBP/DBP [35]. A study included 275 participants from rural district of Shandong Province of China indicated rs820430 functioned as an enhancer of *SLC4A7* [13]. Besides, NBCn1 promotes arterial remodeling which can cause luminal narrowing and obstruct blood flow [36]. It is known to us that increased vascular resistance[37], invasion of placental trophoblast, and failed remodeling of uterine spiral arterial[38] are pathological bases of PIH. Therefore variants of *SLC4A7* might associate with the onset of PIH

through several pathways such as BP regulation, and remodeling of arterial. We reported the polymorphisms of *SLC4A7* rs820430 was associated with the onset of GH and PE in Chinese Han women for the first time. AA genotype at rs820430 is a risk genotype for GH and PE, and A allele at rs820430 is a risk allele for GH. However, more studies are needed to identify pathophysiological path from PIH associated genetic variation, to gene expression, and subsequently to gene function of *SLC4A7* that alters cellular behavior.

CYP21A2 is located on chromosome 6 within the *HLA* (human leukocyte antigen) locus. It encodes 21-hydroxylase enzyme, a member of the cytochrome P450 (CYP450) superfamily [39]. 21-hydroxylase enzyme is necessary to the synthesis of cortisol and aldosterone, the latter regulates the amount of salt retained by the kidneys and then affects blood volume of body and BP. The mutation of *CYP21A2* is related to congenital adrenal hyperplasia (CAH) [40]. Many studies have investigated the consequences of the mutations of the CYP450 superfamily. Though Chatuphonprasert et al. found the association between PE and altered expression of CYP27A1 protein in placenta, maternal, and/or fetal serum [41], another research conducted in a Korean population detected no significant association between polymorphisms in *CYP11A1* gene and PE [42]. Also, a Spanish study included 500 Caucasians from the region of Asturias indicated that the variation in *CYP3A5*, *CYP3A4*, and *CYP21A2* did not contribute to the risk for PIH [43]. Similarly, in our study no significant association has been observed between variants of *CYP21A2* rs2021783 and PIH.

Our case-control study was limited by the small sample size. It is difficult to identify very weak associations between SNP and PIH. This issue is common in gene-association studies. Although we did not detect significant difference between fetal sex and PIH, the proportions of female fetus in GH and PE groups were higher than those in control group. Different from the systematic review [44], we found primipara were less likely to develop PE. It was due to the unequal proportion of primipara and multipara in our study. In order to detect accurate relations, we still set fetal sex and primipara as covariates in multiple logistic regression. Another limitation of our study is that the gestational age of PE onset and medical regimens were not regularly recorded in the medical records of our study region, we did not conduct subgroup analyses according to early- or late-onset PE and did not categorize PE cases by severity. Also, we did not adjust for family history of hypertension or PIH and pre-pregnancy body mass index (BMI) in the multiple logistic regression because the proportion of missing data for it was larger than 15%. Further researches with larger sample size and more comprehensive clinical characteristics are necessary to investigate the disease-related genes in GH and different types of PE.

Conclusions

In conclusion, PIH and hypertension may share genetic factors, which provide potential genetic evidence for patients with PIH having a higher risk for hypertension. Our study reported for the first time that the associations between variants of *MED13L* rs11067763 and GH and between variants of *SLC4A7* rs820430 and GH/PE were significant. Further studies are needed to uncover the molecular mechanisms of these associations.

Abbreviations

Pregnancy-induced hypertension, (PIH); gestational hypertension, (GH); preeclampsia, (PE); single nucleotide polymorphism, (SNP); Genome-wide association studies, (GWAS); mediator complex subunit 13-like (*MED13L*); solute carrier family 4 member 7, (*SLC4A7*); cytochrome P450 family 21 subfamily A member 2, (*CYP21A2*); systolic blood pressure, (SBP); diastolic blood pressure, (DBP); cerebrovascular disease, (CVDs); body mass index, (BMI); angiotensinogen, (AGT); angiotensin II receptor type 1 (AGTR1); gestational diabetes mellitus, (GDM); activating

transcription factor 4, (ATF4); vascular smooth muscle cells, (VSMCs); human leukocyte antigen, (HLA); congenital adrenal hyperplasia, (CAH)

Declarations

Ethics approval and consent to participate

The study was reviewed and approved by the Committee for Human Research Protections of Xiang-Ya School of Medicine. Informed consent was obtained from each individual included in the study, after which blood samples and medical records were collected.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Hongzhuan Tan designed and directed the implementation of the study. Ying Zhou conducted the literature review, statistical analysis, and drafted the manuscript. Xun Li contributed to statistical analyses and interpreting the results. Shimin Hu conducted the genotyping and genetic data analyses. Shujin Zhou designed the medical part of the study protocol and supervised the field activities (participant inclusion / exclusion and data collection).

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Tables

Table 1. Demographic and clinical characteristics of the study groups.

Characteristics	Control	GH	P^a	PE	P^b	P^c
	N=192	N=84		N=108		
Female (n/N (%))	87/188(46.28)	46/75(61.33)	0.027	59/103(57.28)	0.073	0.587
White (n/N (%))	161/192(83.85)	65/84(77.38)	0.199	79/108(73.15)	0.026	0.502
Gestational age at delivery, week,	39(38,40)	39(38,40)	0.581	38(37,39)	<0.001	0.005
BP, mmHg(M(QL,QU))	110(100,120)	138(120,143)	<0.001	140(130,148)	<0.001	0.003
BP, mmHg(M(QL,QU)) ^d	70(60,75)	85(78,92)	<0.001	89(80,95)	<0.001	0.084
Glucose, mg/dL (M(QL,QU))	61.65(50.63,69.06)	61.77(58.04,69.99)	0.129	67.41(60.37,73.95)	<0.001	0.013
Uric acid, umol/L (mean ± SD)	304.89±99.40	360.06±97.51	<0.001	395.03±105.86	<0.001	0.042
Weight, kg (mean ± SD)	3.27±0.41	3.31±0.50	0.494	3.12±0.68	0.048	0.036

Abbreviations: GH, gestational hypertension; PE, preeclampsia; M (QL, QU), Median (lower quartile (25%), upper quartile (75%)); SBP, systolic blood pressure; DBP, diastolic blood pressure.

a Comparison of GH and control group.

b Comparison of PE and control group.

c Comparison of GH and PE group.

d In-hospital SBP and In-hospital DBP: BP were measured when pregnant women came to hospital for delivery and before entering the delivery room. GH and PE women might have received treatment to control their BP before measurements.

Table 2. Distributions of tested alleles and genotypes among cases and control subjects.

Gene	SNP	Genotype/ Allele	Controls		GH		<i>P</i>	PE		<i>P</i>
			n	%	n	%		n	%	
<i>MED13L</i>	rs11067763	AA	72	38.9	29	35.8	0.007	44	41.9	0.091
		AG	71	38.4	45	55.6		48	45.7	
		GG	42	22.7	7	8.6		13	12.4	
		A	215	58.1	103	63.6	0.250	136	64.8	
		G	155	41.9	59	36.4		74	35.2	
<i>SLC4A7</i>	rs820430	GG	82	44.3	32	38.6	0.052	48	45.3	0.053
		GA	94	50.8	40	48.2		45	42.5	
		AA	9	4.9	11	13.3		13	12.3	
		G	258	71.5	104	62.7	0.043	141	66.5	
		A	103	28.5	62	37.3		71	33.5	
<i>CYP21A2</i>	rs2021783	CC	108	56.2	50	59.5	0.884*	54	50.0	0.200
		CT	66	34.4	26	31.0		38	35.2	
		TT	16	8.3	8	9.5		16	14.8	
		C	282	74.2	126	75.0	0.845	146	67.6	
		T	98	25.8	42	25.0		70	32.4	

**P* value was calculated by Fisher’s exact test.

Table 3. Logistic regression for SNPs.

NP	Genotype/ Allele	Univariate logistic regression model				Multiple logistic regression model			
		GH		PE		GH		PE	
		OR	95%CI	OR	95%CI	OR*	95%CI*	OR*	95%CI*
13L	G vs. A	0.795	(0.543,1.163)	0.755	(0.532,1.072)	0.830	(0.523,1.474)	0.718	(0.499,1.033)
067763	AG vs. AA	1.574	(0.890, 2.783)	1.106	(0.655, 1.869)	1.598	(0.864, 2.959)	0.982	(0.570,1.691)
	GG vs. AA	0.414	(0.167, 1.027)	0.506	(0.245, 1.047)	0.472	(0.184, 1.212)	0.497	(0.237,1.045)
13L	AG+GG	1.143	(0.665,1.964)	0.883	(0.543,1.438)	1.165	(0.655,2.074)	0.798	(0.483,1.320)
067763	vs. AA								
inant									
13L	GG vs.	0.322	(0.138,0.752)	0.481	(0.245,0.945)	0.376	(0.158,0.892)	0.489	(0.245,0.979)
067763	AA+AG								
sive									
A7	A vs. G	1.373	(0.935,2.018)	1.160	(0.808,1.665)	1.560	(1.037,2.347)	1.278	(0.879,1.857)
0430	AG vs. GG	1.090	(0.628,1.892)	0.818	(0.494, 1.353)	1.305	(0.724, 2.354)	0.881	(0.523,1.484)
	AA vs. GG	3.132	(1.186,8.271)	2.468	(0.982,6.201)	4.367	(1.494,12.763)	2.931	(1.134,7.574)
A7	AG+AA	1.269	(0.748,2.153)	0.962	(0.595, 1.555)	1.531	(0.867, 2.704)	1.065	(0.647, 1.752)
0430	vs. GG								
inant									
A7	AA vs.	2.988	(1.188,7.517)	2.734	(1.127, 6.632)	3.562	(1.343, 9.447)	3.351	(1.352, 8.303)
0430	GG+AG								
sive									
1A2	T vs. C	0.959	(0.631,1.457)	1.380	(0.957,1.990)	0.874	(0.561,1.363)	1.304	(0.891,1.908)
01783	CT vs. CC	0.851	(0.484, 1.496)	1.152	(0.688, 1.929)	0.745	(0.409, 1.359)	1.103	(0.647, 1.881)
	TT vs. CC	1.080	(0.434, 2.690)	2.000	(0.930, 4.303)	0.973	(0.370, 2.557)	1.389	(0.919, 2.100)
1A2	CT+TT vs.	0.896	(0.532, 1.509)	1.317	(0.820, 2.116)	0.789	(0.454, 1.371)	1.239	(0.758, 2.023)
01783	CC								
inant									
1A2	TT vs.	1.145	(0.470, 1.891)	1.891	(0.905, 1.087)	1.087	(0.425, 2.781)	1.330	(0.902, 1.891)

1783	CC+CT	2.789)	3.954)	1.960)
sive				

Abbreviations: GH, gestational hypertension; PE, preeclampsia; SNP = single nucleotide polymorphism; OR = odd ratio; CI = confidence interval.

* Statistics estimated by multiple logistic regression analysis after adjusting for fetal sex and primipara.