The Common Single-Nucleotide Polymorphism rs2681472 Is Associated With Early-Onset Preeclampsia in Northern Han Chinese Women

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Abstract

Preeclampsia, characterized by hypertension and proteinuria, remains a leading cause of maternal morbidity and mortality. Recently, a genome-wide association study (GWAS) identified the single-nucleotide polymorphism, rs2681472, as a new hypertension susceptibility genetic variant. The purpose of this study was to evaluate the association between preeclampsia and rs2681472 in a Northern Han Chinese population. We genotyped 1218 unrelated Northern Han Chinese women, including 515 patients with preeclampsia and 703 healthy controls. No significant differences were detected in the allele frequencies between patients and controls ($P = .23$). When patients were divided into early-onset and late-onset preeclampsia according to gestational age of disease onset, the allele frequencies significantly differed between controls and patients with early-onset preeclampsia ($P = .02$). Genotype frequencies also were significantly different between controls and patients early-onset preeclampsia when data were analyzed under additive ($P = .03$) and dominant ($P = .009$) models. We replicated this association in an independent Northern Han Chinese population and observed a significant difference in the allele frequencies between patients with early-onset preeclampsia and controls ($P = .011$). We report that rs2681472 is associated with early-onset preeclampsia in Northern Han Chinese women.

Keywords

preeclampsia, genetics, ATP2B1, polymorphisms

Introduction

Preeclampsia, occurring in 3% to 5% of all pregnancies, is a major cause of preterm birth, intrauterine growth restriction, and stillbirth and increases the maternal risk of eclampsia; hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome; renal failure; pulmonary edema; stroke; and death. The exact etiology of preeclampsia is unknown, but a genetic predisposition is thought to play a crucial role in its development. The estimated heritability of preeclampsia is 54%.¹

Recently, a genome-wide association study (GWAS) that included a large cohort of Europeans identified the single-nuclear polymorphism (SNP) rs2681472, near *ATP2B1*, as the only genetic variant associated with hypertension (odds ratio [OR] = 1.17, $P = 1.7 \times 10^{-8}$).² Numerous subsequent studies have evaluated the role of the *ATP2B1* gene in the pathogenesis of hypertension among various ethnic populations.³⁻¹⁰ A large-scale meta-analysis of East Asian populations confirmed a significant association between rs2681472 and hypertension.¹¹ In

the present study, we considered rs2681472 as a genetic risk marker for preeclampsia and evaluated its distribution in 2 independent Northern Han Chinese case-control cohorts.

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Table 1. Probes and Primers of the SNP rs2681472.

SNP	Probes	Primers
rs2681472	FAM-TCTGCCATGTAAATAG-MGB VIC-TCTGCCATGTAAACAG-MGB	F-GAGTTGCAATCTGATGGTTCATAGTG R-GCATTCTCGTTTTGCTTGGA

Materials and Methods

Patients

This study was approved by the Institutional Review Board of Provincial Hospital Affiliated to Shandong University. Each participant provided written informed consent. We conducted a 2-stage study to identify the association between rs2681472 and preeclampsia in a Northern Han Chinese population. The first stage consisted of 1218 unrelated Northern Han Chinese pregnant women who had been recruited from Provincial Hospital Affiliated to Shandong University from December 2009 to November 2011. The second stage of the replication study included 166 patients with early-onset preeclampsia and 178 controls recruited from January 2012 to March 2013. The following information was collected: blood pressure, maternal age, gestational age, fetal weight, prepregnancy body mass index (pre-BMI), and parity. Preeclampsia was defined as the presence of hypertension (blood pressure values $\geq 140/90$ mm Hg at 2 measurements occurring ≥ 6 hours apart) in combination with proteinuria (24-hour urinary protein ≥ 300 mg or urine dipstick protein $\geq ++$) after the 20th week of pregnancy in a previously normotensive woman.¹² Women with preeclampsia were further divided into early-onset and late-onset subgroups according to gestational age of disease onset. Early-onset preeclampsia was defined as occurring before 34 gestational weeks and late-onset preeclampsia developed at or beyond 34 weeks. Controls had normal blood pressure, lacked antenatal medical or obstetric complications, and presented normal fetal growth at delivery (defined as birth weight between the 10th and 90th percentiles at ≥ 37 weeks of gestation). Participants were excluded for the following reasons: chronic hypertension; multiple pregnancies; previous renal, autoimmune, metabolic, or cardiovascular disease; and loss to follow-up. Women with gestational hypertension, defined as elevated blood pressure without proteinuria, were also excluded from this study.

DNA Extraction and Genotyping

Maternal venous whole blood (5 mL) was drawn into EDTA-containing tubes and stored at -80°C . Genomic DNA was extracted using a QIAamp DNA mini kit (Qiagen, Hilden, Germany). Genotyping for the *ATP2B1* rs2681472 SNP was performed using a TaqMan-MGB probe assay (Invitrogen Trading, Shanghai, China; Table 1). Reactions were carried out on a Roche LightCycler 480 with the following program: pre-incubation at 95°C for 4 minutes followed by 42 cycles of denaturation at 95°C for 15 seconds, annealing, extension, and detection for 40 seconds at 60°C . Randomly selected samples

(5%) were further genotyped by direct sequencing to validate the genotyping assays.

Statistical Analysis

The basic characteristics of the patients were expressed as mean $\pm$ standard deviation. Concordance with Hardy-Weinberg proportions was tested using Haploview software (Broad Institute, Cambridge, Massachusetts). The chisquare test was performed to compare allele frequencies. Genetic models were divided into additive (+/+ vs +/- vs -/-), dominant (+/+ plus +/- vs -/-), and recessive (+/+ vs +/- plus -/-) groups and then were analyzed by 1-way analysis of variance. Unconditional logistic regression analysis was used to adjust for parity, pre-BMI, and maternal age using SPSS version 17.0 software (SPSS Inc, Chicago, Illinois). $P < .05$ was regarded as statistically significant.

Results

The clinical characteristics of the participants are summarized in Table 2. No significant differences were detected regarding parity between patients and controls ($P > .05$). The mean maternal age and pre-BMI were higher among patients than controls ($P < .05$). The gestational age at birth and fetal weight were significantly lower among patients than controls ($P < .05$).

The allele frequencies of the participants showed no deviation from Hardy-Weinberg equilibrium. As shown in Table 3, we observed no difference in the allele frequencies between preeclamptic women and controls ($P = .23$; OR = 1.11; 95% confidence interval [CI] = 0.94-1.31, Table 3). When early- and late-onset preeclampsia subgroups were considered, the allele frequencies were significantly different between early-onset preeclampsia and controls ($P = .02$; OR = 1.29; 95% CI = 1.04-1.59, Table 4). In contrast, no significant difference was observed between patients with late-onset preeclampsia and controls (data not shown).

Genotype frequencies were further analyzed under additive, dominant, and recessive models. No significant differences were detected between patients with preeclampsia and controls under additive ($P = .193$, Table 3), dominant ($P = .09$, Table 3), or recessive models ($P = .94$, Table 3). However, we detected a significant difference between patients with early-onset preeclampsia and controls under additive ($P = .03$, Table 4) and dominant models ($P = .009$, Table 4).

A total of 344 unrelated Northern Han Chinese women were included in the replication study. We observed a significant difference in the allele frequencies between early-onset preeclampsia and controls ($P = .011$, Table 5). There was also a

Table 2. Characteristics of Women With and Without Preeclampsia.

Characteristic	First Stage Study		Replication	
	Controls (N = 703)	PE (N = 515)	Controls (N = 178)	Early-onset (N = 166)
Maternal age	28.5 ± 4.9	29.8 ± 6.0	27.8 ± 3.9	30.7 ± 3.5
Pre-BMI	21.9 ± 3.2	24.3 ± 3.7	22.6 ± 3.2	24.4 ± 3.3
Primiparas	365 (51.9%)	276 (53.6%)	77 (43.3%)	81 (48.8%)
SBP, mm Hg	119.4 ± 10.1	160.2 ± 19.3	117.7 ± 9.4	158.4 ± 19.9
DBP, mm Hg	77.1 ± 7.4	108.2 ± 14.2	74.9 ± 6.9	105.9 ± 14.5
Delivery weeks	39.2 ± 1.4	35.7 ± 3.5	39.2 ± 1.2	29.0 ± 6.1
Fetal weight, g	3413.3 ± 473.2	2622.9 ± 928.5	3335.4 ± 368.0	1654.7 ± 814.7

Abbreviations: PE, preeclampsia; pre-BMI, prepregnancy body mass index; SBP, systolic blood pressure; DBP, Diastolic blood pressure.

Table 3. Genotype and Allele Frequencies of rs2681472 in Women With and Without Preeclampsia in First Stage Study.

Model	Control (N = 703)	Preeclampsia (N = 515)	P	OR (95% CI)
Additive			.193	NA
CC	89	66		
CT	334	219		
TT	280	230		
Dominant			.09	1.22 (0.97-1.54)
CC + CT	423	285		
TT	280	230		
Recessive			.94	0.99 (0.70-1.39)
CC	89	66		
CT + TT	614	449		
Allele			.23	1.11 (0.94-1.31)
C	512	351	.30 ^a	
T	894	679		

Abbreviations: OR, odds ratio between case and control group; CI, confidence interval; NA, not applicable.

^a P value was adjusted for maternal age, prepregnancy body mass index, and primiparity.

Table 4. Genotype and Allele frequencies of rs2681472 in Women With Early-Onset Preeclampsia and Controls in First Stage Study.

Model	Control (N = 703)	Early-onset PE (N = 260)	P	OR (95% CI)
Additive			.032	NA
CC	89	28		
CT	334	104		
TT	280	128		
Dominant			.009	1.47 (1.10-1.95)
CC + CT	423	132		
TT	280	128		
Recessive			.425	1.20 (0.77-1.89)
CC	89	28		
CT + TT	614	232		
Allele			.02	1.29 (1.04-1.59)
C	512	160	.035 ^a	
T	894	360		

Abbreviations: PE, preeclampsia; OR, odds ratio between case and control group; CI, confidence interval; NA, not applicable.

^a P value was adjusted for maternal age, prepregnancy body mass index, and primiparity.

significant difference in genotype frequencies when analyzed under additive ($P = .014$, Table 5) and recessive models ($P = .002$, Table 5).

Discussion

An association between rs2681472 and hypertension was originally reported by a GWAS of hypertension in European populations. This association was subsequently confirmed by a large-scale meta-analysis of East Asian populations. In the present study, no significant difference in rs2681472 was detected between patients with preeclampsia and controls, suggesting that genes associated with hypertension are not necessarily the same genes that cause preeclampsia, even in a single ethnic group. When patients were divided into early-onset and late-onset subgroups, a significant difference in the allele frequency was detected between patients with early-onset preeclampsia and controls. To further validate this finding, we genotyped patients from an independent replication set and

again observed a significant difference between patients with early-onset preeclampsia and controls.

The *ATP2B1* gene, located at 12q21.3, encodes a plasma membrane Ca^{2+} pump (*PMCA1*) belonging to the family of P-type primary ion transport adenosine triphosphatases. This pump plays a crucial role in the fine regulation of intracellular free calcium concentration. Its role in blood pressure regulation has been explored extensively.¹³ A recent report by Kobayashi et al demonstrated a functional role of *ATP2B1* in blood pressure control in vivo.¹⁴ Specifically, mice expressing a vascular smooth muscle cell-specific knockout (KO) of *ATP2B1* expressed significantly lower levels of ATP2B1 messenger RNA and protein in the aorta compared to control mice, and KO mice exhibited higher systolic blood pressures.¹⁴

Preeclampsia is characterized by a disturbance in calcium metabolism that is evidenced by a decreased calcium concentration in plasma and increased calcium concentrations in the cell membrane and cytosol.¹⁵⁻¹⁸ In patients with preeclampsia, the activity of PMCA was reduced by approximately 50% in maternal and neonatal red blood cell ghosts, myometria, and

Table 5. Genotype and Allele frequencies of rs2681472 in Women With Early-Onset Preeclampsia and Controls in Replication Study.

Model	Control (N = 178)	Early-onset PE (N = 166)	P	OR (95% CI)
Additive			.014	NA
CC	31	12		
CT	78	76		
TT	69	78		
Dominant			.123	1.40 (1.91-2.15)
CC +CT	109	88		
TT	69	78		
Recessive			.002	2.90 (1.44-5.85)
CC	31	12		
CT +TT	147	165		
Allele			.011	1.50 (1.10-2.06)
C	140	100	.034 ^a	
T	216	232		

Abbreviations: PE, preeclampsia; OR, odds ratio between case and control group; CI, confidence interval; NA, not applicable.

^a P value was adjusted for maternal age, pre-pregnancy body mass index, and primiparity.

syncytiotrophoblast basal (fetal-facing) plasma membranes compared to normal controls.¹⁹⁻²² The decreased activity of PMCA may be responsible, at least in part, for observed increases in peripheral resistance and blood pressure among patients with preeclampsia.

Numerous studies have indicated that preeclampsia is a syndrome rather than a definitive disease. In this study, rs2681472 was significantly associated with early-onset preeclampsia but not with late-onset preeclampsia. Early-onset preeclampsia is associated with more recurrences than late-onset preeclampsia and is thought to be affected by environmental or genetic factors.²³ Conversely, late-onset preeclampsia is closely associated with a failed adaption of the maternal cardiovascular system to changes in late pregnancy and is regarded as a maternal phenotype.²³

This study evaluated only Han Chinese women so the genetic background of the study population was consistent. However, this study had several limitations. Genetic factors that influence the development of preeclampsia differ by ethnic group, so our observed association between rs2681472 and early-onset preeclampsia needs to be confirmed in a larger sample size composed of various ethnic groups. Second, preeclampsia is thought to be a maternal-fetal disease. We only considered maternal genotype in this study and future studies should focus on the role of fetal or paternal genes in the development of preeclampsia.

We identified a significant association between rs268172 and early-onset preeclampsia in Northern Han Chinese women. Additional studies are warranted to confirm and extend our findings.

Authors' Note

Ji-Peng Wan, Hong Wang, and Chang-Zhong Li contributed equally to this article.

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Declaration of Conflicting Interests

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References

- Salonen Ros H, Lichtenstein P, Lipworth L, Cnattingius S. Genetic effects on the liability of developing pre-eclampsia and gestational hypertension. *Am J Med Genet.* 2000;91(4):256-260.
- Levy D, Ehret GB, Rice K, et al. Genome-wide association study of blood pressure and hypertension. *Nat Genet.* 2009;41(6):677-687.
- Kohara K, Tabara Y, Nakura J, et al. Identification of hypertension-susceptibility genes and pathways by a systemic multiple candidate gene approach: the millennium genome project for hypertension. *Hypertens Res.* 2008;31(2):203-212.
- Cho YS, Go MJ, Kim YJ, et al. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat Genet.* 2009;41(5):527-534.
- Hong KW, Go MJ, Jin HS, et al. Genetic variations in ATP2B1, CSK, ARSG and CSMD1 loci are related to blood pressure and/or hypertension in two Korean cohorts. *J Hum Hypertens.* 2010;24(6):367-372.
- Hong KW, Jin HS, Lim JE, Kim S, Go MJ, Oh B. Recapitulation of two genomewide association studies on blood pressure and essential hypertension in the Korean population. *J Hum Genet.* 2010;55(6):336-341.
- Tabara Y, Kohara K, Kita Y, et al. Common variants in the ATP2B1 gene are associated with susceptibility to hypertension: the Japanese millennium genome project. *Hypertension.* 2010;56(5):973-980.
- Takeuchi F, Isono M, Katsuya T, et al. Blood pressure and hypertension are associated with 7 loci in the Japanese population. *Circulation.* 2010;121(21):2302-2309.
- Ho JE, Levy D, Rose L, Johnson AD, Ridker PM, Chasman DI. Discovery and replication of novel blood pressure genetic loci in the women's genome health study. *J Hypertens.* 2011;29(1):62-69.
- Wang Y, Zhang Y, Li Y, et al. Common variants in the ATP2B1 gene are associated with hypertension and arterial stiffness in Chinese population. *Mol Biol Rep.* 2013;40(2):1867-1873.
- Xi B, Tang W, Wang Q. Polymorphism near the ATP2B1 gene is associated with hypertension risk in East Asians: a meta-analysis

- involving 15 909 cases and 18 529 controls. *Blood Press.* 2012; 21(2):134-138.
12. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. *Obstet Gynecol.* 2002;99(1):159-167.
 13. Tabara Y1, Kohara K, Miki T. Millennium Genome Project for Hypertension. Hunting for genes for hypertension: the millennium genome project for hypertension. *Hypertens Res.* 2012;35(6):567-573.
 14. Kobayashi Y, Hirawa N, Tabara Y, et al. Mice lacking hypertension candidate gene ATP2B1 in vascular smooth muscle cells show significant blood pressure elevation. *Hypertension.* 2012; 59(4):854-860.
 15. Kisters K, Barenbrock M, Louwen F, Hausberg M, Rahn KH, Kosch M. Membrane, intracellular, and plasma magnesium and calcium concentrations in preeclampsia. *Am J Hypertens.* 2000; 13(7):765-769.
 16. Kisters K, Korner J, Louwen F, et al. Plasma and membrane Ca^{2+} and Mg^{2+} concentrations in normal pregnancy and in preeclampsia. *Gynecol Obstet Invest.* 1998;46(3):158-163.
 17. Steinert JR, Wyatt AW, Poston L, Jacob R, Mann GE. Preeclampsia is associated with altered Ca^{2+} regulation and NO production in human fetal venous endothelial cells. *Faseb J* 2002;16:721-3.
 18. Idogun ES, Imarengiaye CO, Momoh SM. Extracellular calcium and magnesium in preeclampsia and eclampsia. *Afr J Reprod Health.* 2007;11(2):89-94.
 19. Nardulli G, Proverbio F, Limongi FG, Marin R, Proverbio T. Preeclampsia and calcium adenosine triphosphatase activity of red blood cell ghosts. *Am J Obstet Gynecol.* 1994;171(5): 1361-1365.
 20. Casart Y, Proverbio T, Marin R, Proverbio F. Comparative study of the calcium adenosine triphosphatase of basal membranes of human placental trophoblasts from normotensive and preeclamptic pregnant women. *Gynecol Obstet Invest.* 2001;51(1): 28-31.
 21. Carreiras MM, Proverbio T, Proverbio F, Marin R. Preeclampsia and calcium-ATPase activity of red cell ghosts from neonatal and maternal blood. *Hypertens Pregnancy.* 2002;21(2):97-107.
 22. Carrera F, Casart YC, Proverbio T, Proverbio F, Marin R. Preeclampsia and calcium-ATPase activity of plasma membranes from human myometrium and placental trophoblast. *Hypertens Pregnancy.* 2003;22(3):295-304.
 23. Hernandez-Diaz S, Toh S, Cnattingius S. Risk of pre-eclampsia in first and subsequent pregnancies: prospective cohort study. *BMJ.* 2009;338:b2255.