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Genetic Thrombophilia Variants and Risk for Preeclampsia Among American Indians

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Abstract

Objective—To determine the prevalence of thrombophilic genetic variants in an American Indian population and determine if they are associated with preeclampsia.

Methods—A total of 87 cases, 165 controls and an additional 75 population-based controls were genotyped for two thrombophilic polymorphisms.

Results—The allelic prevalence of the factor V Leiden and 20210 G/A prothrombin variants in this population was 2.1% and 0.5% respectively. No statistically significant associations between these genetic variants and preeclampsia were found.

Conclusion—The prevalence of thrombophilic variants is of possible public health significance for other morbidity; but perhaps not in relation to preeclampsia.

Keywords

Pre-eclampsia; Genetics; Factor V Leiden; Prothrombin 20210 polymorphism; American Indian

Introduction

Preeclampsia (PE) is a pathologic state of pregnancy thought to begin as a subclinical condition involving abnormal placentation, endothelial dysfunction, altered coagulation and a heightened inflammatory state. The clinical manifestations of hypertension, specific renal pathology and proteinuria generally become apparent after 20 weeks of gestation; and of women so affected, many will develop life-threatening maternal and fetal complications (1). The incidence of PE is frequently reported to be approximately 5% to 8% of pregnancies (2) in most populations; and one report from the Navajo American Indian population found a prevalence of 7.7% (3). The risk is increased by a number of factors, such as maternal age, nulliparity, multifetal gestation, preexisting hypertension or diabetes, and various renal and inflammatory pathologies (4). In addition to recognized environmental and clinical risk

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factors, inherited genetic factors have been demonstrated by increased risk of PE among offspring of those affected by PE, and by specific genetic variants that are associated with risk of PE (5–7).

In 1994, Bertina(8) and Greengard (9) independently reported that a genetic variant of the human factor V gene (*F5*), factor V Leiden (FVL), is often found in patients with thrombophilia. The allelic frequency of FVL is relatively high in European populations (4.4%), much lower in Asians (0.4%) and uncommon among native populations in Africa and the Americas (10). Analysis of haplotypes associated with FVL indicate that this variant may have arisen from a single founder (11) and despite a 40- to 80-fold increased risk for pathologic clot formation among FVL homozygotes (12), in the past it may have conferred a selective advantage on heterozygotes due to reduced blood loss during trauma or childbirth (13).

This emerging understanding of the genetic control of coagulation and the known influence of genetics on PE soon led investigators to study possible associations between of thrombophilia (FVL and other genetic variants) and PE (14,15). Meta-analyses have found significant pooled odds ratios (OR, 1.81; 95% CI, 1.14 to 2.87) for preeclampsia in those with one or more FVL alleles(16); but other studies have not found significant associations (17). In 1996 the prothrombin (*F2*) gene was recognized to have a functional G/A variant at nucleotide 20210 (hereafter abbreviated as “PT”) that increases the level of circulating prothrombin and is associated with an increased risk of thrombosis (18).

Since background genetic influences and other environmental effects may modify the influence of these variants, we investigated the prevalence of thrombophilic variants and their possible association with PE in this American Indian community.

Methods

This study is comprised of Phase I, a case control study and Phase II, a prospective, cohort study. Recruitment for both of these phases was conducted simultaneously, from December 2004 to March 2008. The federally funded Indian Health Service (IHS), through the hospital and clinic located in Belcourt, North Dakota, is the primary health care provider for eligible tribal members of the Turtle Mountain Band of Chippewa. Most potential cases (~80%) were identified by automated query of an electronic medical record database (the Resource, Patient, Management System (RPMS)) at this facility, using a relevant group of ICD9 codes, designed to be inclusive. Additional potential cases (~20%) were “self-identified” among family members and acquaintances during the course of recruiting controls and Phase II participants.

The medical records of all of these potential cases were abstracted for 78 clinically relevant factors, including the highest of up to 3 blood pressure (BP) measures between 20 weeks of gestation and 30 days postpartum and the highest of up to 2 measures of dipstick proteinuria in the same period. Cases were verified as meeting diagnostic criteria for PE if at least 2 of the following were identified:

1. At least three blood pressure values above either 140 systolic or 90 diastolic. In addition, absence of a “prior” (during the year prior to conception and the first 20 weeks of gestation) diagnosis of or treatment for hypertension was required.
2. Proteinuria as indicated by a 24-hour excretion of >300 mg, or at least two +1 dipstick measurements, again in the absence of “prior” proteinuria.
3. A diagnosis of “preeclampsia,” “eclampsia,” or the hemolysis, elevated liver enzymes, low platelet (HELLP) syndrome by an attending physician after 20 weeks of gestation.

These criteria were chosen to be compatible with the NHLBI Working Group on Research on Hypertension during Pregnancy definition(19); but to also consider the clinical judgment of the attending physician.

Phase I controls were ascertained by contact of the first individual to deliver before and after the index case. If a potential control declined participation, the woman delivering during the next day prior or subsequent was contacted; and this was continued until two controls were recruited, one before and one after the index case. This method of ascertaining controls was chosen as a convenient means of randomization and to control for possible seasonal influences on PE(20).

The medical records of all controls were abstracted in the same way as cases; and it was verified that these individuals did not meet criteria for PE.

Birth certificate data was also obtained for all case/control participants to more uniformly ascertain data on such factors as smoking, alcohol intake and educational attainment.

Phase II participants have been screened for diagnoses from the same ICD9 group. The genotypic data for this group (without diagnostic evidence of PE) is presently analyzed solely to provide further information on the population prevalence of FVL and PT variants.

For template DNA, capillary blood samples were collected on “FTA Classic Cards” (Whatman Inc) paper; and four 1.2 mm diameter “pellets” were purified according to the manufacturer's recommendations. For the first 147 participants, a 628 bp amplicon including the R506Q mutation (rs6025) was produced using Invitrogen Taq in 1.5 mM MgCl and the following custom primers (GCACAC CAACATGACACATGTATAC and CAGTACCATCACTGCCGAAGGCAA). A standard Mnl I restriction enzyme digest with polyacrylamide gel-based fragment length determination was used to determine genotype, similar to the method described in Bertina et al.(8) Digestion resulted in wild-type allele fragments of 367bp, 224bp, 37bp; and FVL fragments of 367bp and 261bp. For the remaining 185 participants, an Applied Biosystems Inc (ABI) “Taqman”, realtime PCR, genotyping protocol (ABI assay identifier, C_11975250_10) was implemented in a Bio-Rad Mini-Opticon, 4 color thermocycler. The change was instituted for efficiency of throughput and as an assurance of comparability, genotypes of 7 heterozygotes from the gel-based method were confirmed using the Taqman assay. Control FVL +/− and −/− samples were provided by the Coriell Institute for Medical Research, catalog ID number GM14641 and GM14899, respectively.

The PT genotypes were determined using PCR amplification and restriction fragment analysis as previously described (18). Coriell GM16000 was used as a source for control PT (+/−) template DNA.

All genotyping assays were run with positive and negative controls; and all samples detecting any variant alleles were confirmed in duplicate; and in most cases, at least triplicate assays.

Statistical analysis was carried out using SPSS, version 10.1.0 software. Descriptive statistics report mean (± SD) for continuous variables and proportions with 95% CI for discrete variables. McNemar's Chi square tests (1 degree of freedom) were used for testing differences in proportions of genotypes between cases and matched controls. Conditional logistic regression was used to explore the multivariate association of genotype and other variables with risk of PE. Statistical significance was set at $p \leq 0.05$.

Approval was obtained from both the IHS and University of North Dakota Institutional review boards, the tribal government, and individual, informed consent from each participant.

Results

Of the initially identified 93 potential cases and 186 controls, 87 cases and 165 control participants met diagnostic inclusion criteria. Among accepted cases, 58.3% met all 3 diagnostic criteria, whereas the others met 2 criteria. According to the American College of Obstetricians and Gynecologists' definition (4) of “severe preeclampsia”, 56 (64.4%) of these cases met at least one criterion and 21.8% met 2 or more criteria. None of the qualified controls had a clinical diagnosis of PE; but 28.3% met the BP criteria and a different group of 6.2% met the criteria for proteinuria. On average, we contacted 1.3 individuals before enrolling a control and control infants were born an average of 4.1 days before or after the index, case infant. Phase II has enrolled 75 participants.

Pertinent characteristics of the cases and controls are summarized in Table 1. Significant differences between cases and controls were noted for nulliparity, gestation at first prenatal visit, body mass index (BMI), weight at first prenatal visit, and self-reported smoking during pregnancy. Differences in birth weight of infants and blood pressures were felt to be consequences of PE and the applied diagnostic criteria respectively.

Among the case-control sample, genotyping has identified a total of 11 FVL and 2 PT heterozygous; and no homozygous individuals with either of these variants. Pooling results from both cases and controls gives an allele frequency of 2.2% (95% CI: 0.9–3.5) for FVL and 0.4% (95% CI, 0.0–0.8) for PT. Among the 75 Phase II controls, an additional 3 FVL and 1 PT heterozygotes were identified, thus giving the respective allele frequencies in this population more precisely as 2.1% (95% CI, 1.0 to 3.2) and 0.5% (95% CI, 0.0 to 0.9) respectively.

The genotypic results of these paired cases and controls are found in Table 2. McNemar Chi square analysis shows no significant association of either FVL, PT or a composite of these genotypes with PE.

Results of the univariate conditional logistic regression analysis are shown in Table 3 and confirm frequently cited associations between nulliparous status, maternal obesity, and smoking during pregnancy (4, 21). Analysis of the 56 cases meeting the definition of “severe preeclampsia” and their matched controls continued to show a nonsignificant OR of 1.710 (95% CI; 0.415 to 7.050; $p = 0.458$). Multivariate conditional logistic regression results (table 4) revealed strong independent association with maternal age and a somewhat attenuated one with smoking. Nulliparity and obesity continued to show robust, independent effects.

Discussion

In addition to the genetic results, the present investigation indicates that many of the risk factors, such as maternal age, nulliparity and obesity, associated with PE in other populations are also operative in this American Indian community. Age did not show a univariate relationship to PE, but did show an independent association when adjustment for nulliparity occurred in the multivariate analysis. This is likely due to interaction between these covariates, such that age increases risk of PE, but nulliparity is clearly increased among younger mothers. Special mention needs be made about the apparent protective effect of smoking on PE, which is also seen in this study (although of marginal statistical significance in a multivariate model). Conde-Agudelo et al. (21) noted in a comprehensive meta-analysis of this issue, that despite the reduced risk of PE, “…. the well known adverse effects of cigarette smoking during pregnancy outweigh this benefit.”

The literature contains 5 references to FVL variants among American Indians or related populations. Only 3 FVL heterozygous individuals were found among a total of 1014 Oji-Cree and Pima Indian participants in Ontario, Canada and southwestern United States, respectively (22,23). Ridker et al noted one FVL heterozygote among 80 American Indian health professionals in the United States (24). Two other studies among a total of 167 Greenland Inuit and American Indians residing in California found no FVL alleles (25,26). From the perspective of population genetics, our present allele frequency estimate of approximately 2% and a prevalence of heterozygotes of 4% may well be ascribed to European admixture; but this information remains of clinical and public health importance for health care providers in this community.

Spanish investigators reported the highest population prevalence of heterozygous PT individuals (6.5%) (27), whereas a larger study from northern Europe found a population prevalence of 1.7% (28) In the United States, the frequency of PT heterozygosity was 3.8% among 1,774 controls without thrombosis (29). Our results for the PT variant lack adequate precision to allow direct comparisons; but clearly show the need for clinical awareness of this variant in an American Indian population.

Findings of significant heritability for risk of PE (7,30) initially led to many candidate gene studies, which were the primary epidemiologic method of the time. Early results showed an association between PE and thrombophilic genetic polymorphisms (particularly FVL and PT) (14,31); more recent publications have not been confirmatory (32,33). Recent meta-analyses have found statistically significant odds ratios between 2.24 and 8.32 for severe PE among those heterozygous for FVL and PT variants; but insignificant results for more broadly defined PE (16,34,35). Chappell and Morgan have recently provided a particularly thorough review of our understanding of the genetics of PE and the limitations of the various approaches (36). Illustrating the challenge confronting investigators, the Genetics of Pre-eclampsia (GOPEC) consortium enrolled over 650 women with PE; but failed to identify any statistically significant genetic contributors among the 28 single nucleotide polymorphisms (SNPs) within the 7 genes tested (including the FVL variant in *F5*) using transmission/disequilibrium testing (TDT) (17).

The present study also failed to show an association between PE and either the FVL, the PT or the combination of these variants, both before and after adjusting for important covariates. Given the same prevalence of variant genotypes among the controls, the fact that an additional four variants among the cases would have shown an OR of 3.12 (p=0.029) is evidence that the power of this investigation is similar to positive reports in other populations (15,31). Analysis restricted to severe cases gave similar, unsignificant results, albeit with somewhat reduced power to detect a minimum OR of 3.67.

A limitation of this investigation is the lack of sufficient cases to conduct analyses of more homogeneous subgroups, such as those with severe PE. Strengths of this study include an unbiased ascertainment of cases and controls and a well defined phenotype verified by medical record review. The allelic prevalence results will be of importance to clinicians and public health planners within this community. The lack of association between PE and these genetic variants seen in this unique population provides support for the contention that thrombophilic polymorphisms may be more influential in modifying the severity and course of PE than in initiating the condition.

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References

1. Gammill HS, Roberts JM. Emerging concepts in preeclampsia investigation. Front Biosci. 2007; 12:2403–2411. [PubMed: 17127250]

2. Ilekis JV, Reddy UM, Roberts JM. Preeclampsia—a pressing problem: an executive summary of a National Institute of Child Health and Human Development workshop. Reprod Sci. 2007; 14:508–523. [PubMed: 17959880]

3. Levy MT, Jacober SJ, Sowers JR. Hypertensive disorders of pregnancy in southwestern Navajo Indians. Arch Intern Med. 1994; 154:2181–2183. [PubMed: 7944838]

4. ACOG Committee on Obstetric Practice. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. American College of Obstetricians and Gynecologists. Int J Gynaecol Obstet. 2002; 77:67–75. [PubMed: 12094777]

5. Chesley LC, Cooper DW. Genetics of hypertension in pregnancy: possible single gene control of pre-eclampsia and eclampsia in the descendants of eclamptic women. Br J Obstet Gynaecol. 1986; 93:898–908. [PubMed: 3768285]

6. Ward K, Hata A, Jeunemaitre X, Helin C, Nelson L, Namikawa C, et al. A molecular variant of angiotensinogen associated with preeclampsia. Nat Genet. 1993; 4:59–61. [PubMed: 8513325]

7. Arngrimsson R, Björnsson S, Geirsson RT, Björnsson H, Walker JJ, Snaedal G. Genetic and familial predisposition to eclampsia and pre-eclampsia in a defined population. Br J Obstet Gynaecol. 1990; 97:762–769. [PubMed: 2242360]

8. Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature. 1994; 369:64–67. [PubMed: 8164741]

9. Greengard JS, Sun X, Xu X, Fernandez JA, Griffin JH, Evatt B. Activated protein C resistance caused by Arg506Gln mutation in factor Va. Lancet. 1994; 343:1361–1362.

10. Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. Lancet. 1995; 346:1133–1134. [PubMed: 7475606]

11. Cox MJ, Rees DC, Martinson JJ, Clegg JB. Evidence for a single origin of factor V Leiden. Br J Haematol. 1996; 92:1022–1025. [PubMed: 8616062]

12. De Stefano V, Rossi E, Paciaroni K, Leone G. Screening for inherited thrombophilia: indications and therapeutic implications. Haematologica. 2002; 87:1095–1108. [PubMed: 12368166]

13. Donahue BS, Gailani D, Higgins MS, Drinkwater DC, George AL Jr. Factor V Leiden protects against blood loss and transfusion after cardiac surgery. Circulation. 2003; 107:1003–1008. [PubMed: 12600914]

14. Dizon-Townson DS, Nelson LM, Easton K, Ward K. The factor V Leiden mutation may predispose women to severe preeclampsia. Am J Obstet Gynecol. 1996; 175:902–5. [PubMed: 8885745]

15. Grandone E, Margaglione M, Colaizzo D, Cappucci G, Paladini D, Martinelli P, et al. Thromb Haemost. 1997; 77:1052–1054. [PubMed: 9241730]

16. Lin J, August P. Genetic thrombophilias and preeclampsia: a meta-analysis. Obstet Gynecol. 2005; 105:182–192. [PubMed: 15625161]

17. GOPEC Consortium. Disentangling fetal and maternal susceptibility for pre-eclampsia: a British multicenter candidate-gene study. Am J Hum Genet. 2005; 77:127–131. [PubMed: 15889386]

18. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3-prime-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood. 1996; 88:3698–3703. [PubMed: 8916933]

19. Roberts JM, Pearson G, Cutler J, Lindheimer M. NHLBI Working Group on Research on Hypertension During Pregnancy. Summary of the NHLBI Working Group on Research on Hypertension During Pregnancy. Hypertension. 2003; 41:437–445. [PubMed: 12623940]

20. Bodnar LM, Catov JM, Roberts JM. Racial/ethnic differences in the monthly variation of preeclampsia incidence. Am J Obstet Gynecol. 2007; 196:324–5. e1–5. [PubMed: 17403406]

21. Conde-Agudelo A, Althabe F, Belizán JM, Kafury-Goeta AC. Cigarette smoking during pregnancy and risk of preeclampsia: a systematic review. Am J Obstet Gynecol. 1999; 181:1026–1035. [PubMed: 10521771]

22. Hegele RA, Harris SB, Cao H, Hanley AJ, Zinman B. Factor V Leiden (F5 Q506) and vascular disease in Canadian Oji-Cree. Diabetes Care. 1998; 21:1203.

23. Kohler HP, Boothby M, McCormack L, Knowler WC, Grant PJ. Incidence of Arg506 --> Gln mutation (factor V Leiden) in Pima Indians. Thromb Haemost. 1997; 78:961–962. [PubMed: 9268205]

24. Ridker PM, Miletich JP, Hennekens CH, Buring JE. Ethnic distribution of factor V Leiden in 4047 men and women. Implications for venous thromboembolism screening. JAMA. 1997; 277:1305–1307. [PubMed: 9109469]
25. Gregg JP, Yamane AJ, Grody WW. Prevalence of the factor V-Leiden mutation in four distinct American ethnic populations. Am J Med Genet. 1997; 73:334–346. [PubMed: 9415695]
26. deMaat MPM, Kluft C, Jespersen J. World Distribution of factor V Leiden mutation. Lancet. 1996; 347:58.
27. Souto JC, Coll I, Llobet D, del Río E, Oliver A, Mateo J, et al. The prothrombin 20210A allele is the most prevalent genetic risk factor for venous thromboembolism in the Spanish population. Thromb Haemost. 1998; 80:366–369. [PubMed: 9759610]
28. Rosendaal FR, Doggen CJ, Zivelin A, Arruda VR, Aiach M, Siscovick DS, et al. Geographic distribution of the 20210 G to A prothrombin variant. Thromb Haemost. 1998; 79:706–708. [PubMed: 9569177]
29. Ridker PM, Hennekens CH, Miletich JP. G20210A mutation in prothrombin gene and risk of myocardial infarction, stroke, and venous thrombosis in a large cohort of US men. Circulation. 1999; 99:999–1004. [PubMed: 10051291]
30. Cnattingius S, Reilly M, Pawitan Y, Lichtenstein P. Maternal and fetal genetic factors account for most of familial aggregation of preeclampsia: a population-based Swedish cohort study. Am J Med Genet A. 2004; 130:365–371.
31. Kupferminc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med. 1999; 340:9–13. Erratum in: N Engl J Med 1999; 341:384. [PubMed: 9878639]
32. De Groot CJ, Bloemenkamp KW, Duvekot EJ, Helmerhorst FM, Bertina RM, Van Der Meer F, et al. Preeclampsia and genetic risk factors for thrombosis: a case-control study. Am J Obstet Gynecol. 1999; 181:975–980. [PubMed: 10521764]
33. Kim YJ, Williamson RA, Murray JC, Andrews J, Pietscher JJ, Peraud PJ, et al. Genetic susceptibility to preeclampsia: roles of cytosineto-thymine substitution at nucleotide 677 of the gene for ethylenetetrahydrofolate reductase, 68-base pair insertion at nucleotide 844 of the gene for cystathionine beta-synthase, and factor V Leiden mutation. Am J Obstet Gynecol. 2001; 184:1211–1217. [PubMed: 11349190]
34. Robertson L, Wu O, Greer I. Thrombophilia and adverse pregnancy outcome. Curr Opin Obstet Gynecol. 2004; 16:453–458. [PubMed: 15534439]
35. Dudding TE, Attia J. The association between adverse pregnancy outcomes and maternal factor V Leiden genotype: a meta-analysis. Thromb Haemost. 2004; 91:700–711. [PubMed: 15045131]
36. Chappell S, Morgan L. Searching for genetic clues to the causes of pre-eclampsia. Clin Sci (Lond). 2006; 110:443–458. [PubMed: 16526948]

Table 1

Characteristics of matched cases and controls.

Characteristic	Cases	Controls	p value
Number (N)	87	165	
FVL (+/−) genotype	5 (5.7%)	6 (3.6%)	See table 2
PT (+/−) genotype	1 (1.1%)	1 (0.6%)	See table 2
Age, mean (SD)	23.9 (6.7)	23.9 (5.3)	0.821*
Parity (N, % nulliparous)	58 (66.7%)	70 (42.4%)	0.001**
Gestation at first prenatal visit mean wks from LMP, (SD)	13.1 (8.3)	14.7 (8.8)	0.02
Weight at first prenatal	177.8 +/− 47.9	148.9 +/− 59.2	0.001
Body-Mass index (BMI)	30.5 +/− 6.7	27.7 +/− 6.7	0.001
Gestational diabetes, N (% yes)	10 (11.5%)	12 (7.3%)	0.186
Weeks of gestation at delivery	37.02 +/− 3.88	39.30 +/− 1.92	0.001
Birth weight of infant	3081 +/− 956	3452 +/− 553	0.001
Mother's educational attainment (years of education)	12.1 (1.9)	12.2 (2.1)	0.755
Maternal smoking, N (% yes)	30 (37.0%)	74 (53.2%)	0.01
Maternal smoking (Mean cigarettes smoked)	3.1 (5.7)	4.9 (6.8)	0.01
Maternal alcohol use, N (% yes)	2 (2.5%)	2 (1.4%)	0.683
Mean systolic blood pressure	162.0 +/− 15.8	134.2 +/− 17.0	0.001
Mean diastolic blood pressure	97.4 +/− 9.7	78.9 +/− 10.6	0.001

*Differences between means evaluated with paired t test.

**Differences between discrete variables evaluated with McNemar's Chi square test.

Table 2

Genotype associated with case/control (matched-pair) status.

		Controls			Controls			Controls	
FVL		**+/+**	**+/m**	**PT**	**+/+**	**+/m**	**FVL or PT**	**+/+**	**+/m**
Cases	+/+	150	5	+/+	162	1	+/+	147	6
	+/m	9	1	+/m	2	0	+/m	11	1
Chi sq* = 0.643, p = 0 42				Chi sq = 0.00, p = 1.00			Chi sq= 0.94, p= 0.33		

*McNemar Chi square test

Table 3

Univariate, logistic regression analysis of factors associated with pre-eclampsia.

Characteristic	Odds Ratio	p value
FVL variant genotype	1.77	0.377
PT variant genotype	2.00	0.624
FVL or PT variant genotype	1.80	0.314
Age, years of age at delivery	1.001	0.979
Nulliparity	3.18	0.001
Gestation at first prenatal visit, mean weeks from LMP	0.97	0.123
Weight at first prenatal, per pound of weight	1.01	0.001
Body-Mass index (BMI), Kg/meter2	1.06	0.004
Birth weight of infant, per gram of birth weight	0.999	0.003
Mother's educational attainment, years of education	0.972	0.703
Maternal smoking during pregnancy, current smoking	0.489	0.019
Diagnosis of gestational diabetes in current pregnancy	1.588	0.312

Table 4

Multivariate, conditional logistic regression analysis of factors associated with pre-eclampsia.

Multivariate analyses	OR	P value
FVL or PT	1.280	0.696
Age at delivery	1.098	0.005
Nulliparous	6.334	0.001
BMI	1.057	0.060
Smoking	0.607	0.155