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Genetic Variants, Endothelial Function, and Risk of Preeclampsia Among American Indians

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Abstract

Objective—To determine the prevalence in an American Indian population of genetic variants with putative effects on endothelial function and determine whether they are associated with preeclampsia.

Methods—Five genetic polymorphisms potentially related to endothelial function in the *NOS3, GNB3*, and *DDAH1* genes were genotyped from a total of 101 cases, 198 controls, and an additional 110 population-based controls among an American Indian population.

Results—The minor allele frequencies for *NOS3* (rs1799983, rs3918227), *GNB3* (rs5442), and *DDAH1* (rs10158674, rs233115) among those with and without PE in this population were 25, 10, 5, 11, and 30%, respectively. Although not statistically significant, the maximum risk associated with any of these SNPs was 2.22 (0.734–6.73, 95% CI, $p = 0.156$) in a multivariate analysis of the A allele of the rs233115 SNP incorporated in a recessive model.

Conclusion—Although endothelial dysfunction likely plays a role in the pathophysiology of PE, this study was unable to find evidence for an association between these five SNPs on three genes influencing endothelial function and PE. This may be due to insufficient power to detect an association, investigation of SNPs without linkage to risk of PE in this population or other factors. Investigation of additional SNPs in these or related genes and other populations seems warranted.

Keywords

American Indian; Endothelial dysfunction; Genetics; Preeclampsia

INTRODUCTION

Preeclampsia (PE) is a pregnancy-specific condition associated with hypertension and proteinuria manifesting in the second half of pregnancy. Although signs and symptoms present later in pregnancy, the pathologic state of PE is thought to begin as a subclinical condition involving abnormal placentation, endothelial dysfunction, altered coagulation, and

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

a heightened inflammatory state in the early weeks of gestation. The consequences of PE include potentially life-threatening maternal and fetal complications in the acute phase (1) and increased risk of cardiovascular disease later in life (2). The incidence of PE is frequently reported to be approximately 5–8% of pregnancies (3) in most populations. Leeman and Leeman (4) reported a PE rate of 14.5% among Zuni (Pueblo)-Ramah (Navajo) American Indians. Levy et al. (5) reported a prevalence of 7.7% in the Navajo American Indian population (5). The risk for PE is increased by factors such as maternal age, nulliparity, multifetal gestation, pre-existing hypertension, or diabetes, in addition to various renal and inflammatory pathologies (6).

A familial predisposition to PE implicates inherited genetic factors (7,8), and specific genetic variants have been associated with risk of PE (9,10).The etiology of PE is unknown, although endothelial dysfunction as a systemic response to placental insufficiency is well accepted (11,12). Polymorphisms in genes encoding proteins involved in endothelial production and regulation of nitric oxide (NO) have been the target of investigations; however, no susceptibility genes have been clearly identified to date (13). Single nucleotide polymorphisms (SNPs) involving endothelial function were the focus of this study as putative genetic targets for the development of PE. NO is synthesized in the endothelial cells through *NOS3*-catalyzed conversion of L-arginine and is linked to the biological activity of endothelium-derived relaxing factor. *DDAH1* specifies an enzyme that produces asymmetric dimethylarginine, which has an inhibitory effect on *NOS3*. The *GNB3* gene encodes the β-3 subunit of the G protein, involved in the activation of G-protein signaling. Because background genetic influences and other environmental effects may modify the influence of these variants, we investigated the prevalence of variants within genes known to influence endothelial function and their possible association with PE in this American Indian community.

METHODS

This study comprised 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 August 2009. The federally funded Indian Health Service (IHS), through the hospital and clinic located in Belcourt, North Dakota, is the primary healthcare 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 potential cases were abstracted for 78 clinically relevant factors, including the highest of up to three blood pressure (BP) measures between 20 weeks of gestation and 30 days postpartum and the highest of up to two measures of dipstick proteinuria in the same period. Cases were verified as meeting diagnostic criteria for PE if at least two of the following were identified:

1. At least three BP values above either 140 mmHg systolic or 90 mmHg diastolic. In addition, absence of a prior (during the year before conception and the first 20 weeks of gestation) diagnosis of or treatment for hypertension.
2. Proteinuria as indicated by a 24-h excretion of >300 mg, or at least two +1 dipstick measurements in the absence of prior proteinuria.
3. A diagnosis of PE, eclampsia, or 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 (14) and 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 prior or subsequent day 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 (15).The medical records of all controls were abstracted in the same way as cases. It was verified that these individuals did not meet criteria for PE. Birth certificate data were 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 were screened for diagnoses from the same PE case ICD9 codes. The genotypic data for this group (without diagnostic evidence of PE) contributed only to the analysis of population prevalence of these SNPs shown in Table 1.

Template DNA was provided by capillary blood samples collected on “FTA Classic Cards” (Whatman Inc., Clifton, NJ, USA) paper for the majority of participants. Three 1.2 mm diameter “pellets” were punched from the cards and processed according to the manufacturer’s recommendations. For the most recently recruited 10 participants, template DNA was collected and processed using salivary samples and the Oragene (DNA Genotek Inc., Kanata, Ontario, Canada) system.

Pre-designed “TaqMan” (Applied Biosystems Inc.) genotyping assays and protocol were implemented for these SNPs on a real-time, Mini-Opticon (Bio-Rad Laboratories Inc.), four-color thermocycler. Controls were identified for at least two of the three possible genotypes (and “blank” controls) for each SNP and included with each analysis. In the case of rs3918227, HapMap genotypes (16) provided all three control genotypes for samples obtained from the Coriell Institute for Medical Research. These genotypes were confirmed in our laboratory on three separate analyses. Homozygous “A” allele control material could not be identified for the rs5442 SNP; however, internal controls for the other two genotypes gave consistent results. Samples detecting the more infrequent genotypes were generally duplicated in a minimum of two assays. Genotyping was robust, with only five samples failing analysis in over three attempts among 1689 final genotypes determined. The number of pairs analyzed varied by SNP (as noted in Table 4) as primer reagents could only be

ordered in minimal volumes sufficient for about 375 samples, and it was not cost-efficient to reorder primers for perhaps an additional 15 or 20 samples.

Statistical analysis was primarily carried out using SPSS version 10.1.0 software, with Egret version 2.0.31 used for the logistic regression analysis. Descriptive statistics report mean (±SD) for continuous variables and proportions with 95% CI for discrete variables. Hardy–Weinberg analysis was based on standard chi-square methods. 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 and the tribal government. Individual informed consent was obtained from each participant.

RESULTS

Among the 101 cases, 54.9% met all three diagnostic criteria, whereas (by definition) all others met two criteria. According to the American College of Obstetricians and Gynecologists' definition (6) of "severe pre-eclampsia," 76 (74.5%) of these cases met at least one criterion and 18 (17.6%) met two or more criteria. None of the 198 qualified controls had a clinical diagnosis of PE; but 55 (27.9%) met the BP criteria and a different group of 14 (7.1%) met the criteria for proteinuria. Control infants were born an average of 5.4 (range 0–44) days before or after the index case infant.

Table 1 summarizes the SNPs tested, population prevalences, and consistency with Hardy–Weinberg equilibrium among cases, controls, and the prospective cohort.

Pertinent characteristics of the cases and controls are shown in Table 2. The prevalence of SNPs in genes encoding endothelial NO synthase (*NOS3*), dimethylarginine dimethylaminohydrolase (*DDAH1*), and G-protein β (*GNB3*) were not significantly different in cases versus controls. Significant differences between cases and controls were noted for nulliparity, gestation at first prenatal visit, body mass index (BMI), weight at first prenatal visit, selfreported smoking during pregnancy, and both systolic and diastolic BP. Differences in near-term delivery, birth weight of infants, and BPs were likely consequences of PE and the applied diagnostic criteria, respectively.

The genotypic results of paired cases and controls are found in Table 3. McNemar chi-square analysis of pairwise comparisons (majority allele dominant, minor allele dominant) fails to show any significant associations.

Results of the univariate conditional logistic regression analysis are shown in Table 4 and confirm frequently cited associations between nulliparous status, maternal obesity, and birth weight (6,17). Multivariate conditional logistic regression results (Table 5) continued to show robust, independent effects of nulliparity and obesity. After adjustment for nulliparity in the multivariate model, increasing maternal age becomes a significant risk factor for PE.

Analysis of the 76 cases meeting the definition of severe PE and their 147 matched controls did not change any of the previously mentioned univariate relationships, except for the effect of gestational diabetes, which showed an odds ratio of 2.75 ($p = 0.028$). There were no cases with possibly confounding, pre-existing renal or auto-immune disease. Multivariate conditional logistic regression results continued to show a strong independent association between nulliparity and obesity, but a somewhat attenuated relation to maternal age ($p = 0.068$). Gestational diabetes was not an independent risk factor after multivariate adjustment.

DISCUSSION

The increased risk for PE within families suggests the potential for heritable genetic factors (8,13,18,19). The identification of susceptibility genes in the heterogenous disorder of PE is particularly complex, yet could result in therapeutic targets with the potential to reduce risk for PE across generations. Findings of significant heritability for risk of PE (8,20) initially led to many candidate gene studies, which were the primary epidemiologic method of the time. 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 (13). Illustrating the challenge confronting investigators, the Genetics of Pre-eclampsia Consortium (GOPEC) enrolled over 650 women with PE, but failed to identify any statistically significant genetic contributors among the 28 SNPs within the seven genes tested using transmission/disequilibrium testing (21).

Genes associated with the regulation of NO represent unique candidates for investigation, as reduced NO production is implicated in the endothelial dysfunction associated with PE (22–24). Racial and ethnic differences in biologic regulation of NO-dependent vasodilation have been described (25); however, genetic influences regulating NO balance in the American Indian population have not been characterized. In this study, we investigated SNPs within genes associated with endothelial function as putative targets for the development of PE among American Indian women from the Turtle Mountain Band of Chippewa.

The prevalence of SNPs in the analyzed genes of *NOS3, GNB3* and *DDAH1* was not significantly different in cases as compared to controls. Further analysis using McNemar chi-square testing also showed no association between various models of genetic effect and these SNPs. Likewise, univariate and multivariate conditional logistic regression analysis indicated the absence of association with PE. Our findings were consistent with those of Lade et al. (26) and Kim et al. (27), finding no association of SNPs in *NOS3* or DDAH with PE. In contrast, a recent meta-analysis showed a mild increased risk associated with the T recessive genotype of rs1799983 (10); and Akbar et al. (28) reported a total of eight SNPs in the *DDAH1* gene, with four common *DDAH1* haplotypes associated with PE in a case–control study of Finnish women. Of the SNPs examined in this study, in either univariate or adjusted models, the 3′ UTR variant of *DDAH1* (rs233115) yielded the highest estimated odds ratio, and Akbar et al. (28) also found the strongest association signals in the 3′ UTR of this gene. Jansen et al. (29) investigated the T allele (825T) of a SNP in the *GNB3* gene associated with endothelial dysfunction in Dutch women, finding no association with PE. Our results are in agreement, suggesting that this SNP does not contribute to PE among American Indian women.

The SNPs chosen for this study may not be functional in the sense of affecting the coding or expression of proteins that can influence the pathogenesis of PE and may not even be in linkage disequilibrium with risk variants of these genes. Although *post hoc* sensitivity analysis suggests the power of this study is adequate to detect minimum odds ratios of 1.7 for some SNPs and models, genetic risk variants in these genes may have lesser effect sizes, and/or act in a multifactorial manner.

In addition to the genetic results, this 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 (17,30–32). Furthermore, the effect of PE on near-term delivery and low birth weight are consistent with expected sequelae of this complex syndrome. 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.

A limitation of this investigation is the lack of sufficient cases to conduct analyses of more homogeneous subgroups, such as those limited to nulliparous pregnancies. 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 could be of importance to clinicians and public health planners within this community.

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Table 1

Characteristics of SNPs studied and population prevalences.

Gene	dbSNP ID	Functional effect	Minor allele frequency	95% CI	Hardy–Weinberg (*p*-value)
NOS3	rs1799983	E298D	T = 25.3%	21.9–28.8%	0.45
NOS3	rs3918227	INTRON	A = 9.79%	7.4–12.2%	0.064
GNB3	rs5442	S272G	A = 5.01%	3.5–6.7%	0.32
DDAH1	rs10158674	syn A187A	C = 11.05%	8.9–13.3%	0.70
DDAH1	rs233115	3′ UTR	A = 30.06%	26.6–33.5%	0.85

Table 2

Characteristics of matched cases and controls.

Characteristic	Cases	Controls	*p*-value
Number (*N*)	101	198	
NOS3, rs1799983, T allele freq	55/198 = 0.28	96/382 = 0.25	0.556
NOS3, rs3918227, A allele freq	17/184 = 0.09	34/310 = 0.11	0.647
GNB3, rs5442, A allele freq	9/196 = 0.05	22/368 = 0.06	0.621
DDAH1, rs10158674, C allele freq	22/182 = 0.12	38/378 = 0.10	0.560
DDAH1, rs233115, A allele freq	61/202 = 0.30	117/392 = 0.30	0.995
Age, mean years (SD)	24.12 (6.52)	24.01 (5.41)	0.855
Parity (*N*, % nulliparous)	67 (66.2)	80 (40.4)	<0.001 †
Gestation at first prenatal visit mean weeks from LMP (SD)	11.78 (7.00)	13.48 (7.73)	0.026
Weight (lbs) at first prenatal	180.9 (45.1)	162.3 (38.1)	<0.001
Body mass index (BMI)	30.59 (6.97)	27.65 (6.43)	<0.001
Gestational diabetes, *N* (% yes)	13 (12.9)	12 (6.1)	0.170
Weeks of gestation at delivery	36.91 (3.98)	39.27 (1.96)	<0.001
Birth weight of infant (grams)	3041 (966.1)	3452 (584.3)	<0.001
Mother's educational attainment (years of education)	12.02 (1.81)	12.29 (2.21)	0.203
Maternal smoking, *N* (% yes)	38/95 (40.0)	90/185 (48.6)	0.193
Maternal smoking (mean cigarettes smoked)	3.39 (5.68)	4.80 (6.81)	0.034
Mean systolic blood pressure (mmHg)	162.3 (17.3)	134.1 (16.0)	<0.001
Mean diastolic blood pressure (mmHg)	97.1 (10.1)	79.0 (9.9)	<0.001

* Differences between means evaluated with paired *t* test.

† Differences between discrete variables evaluated with McNemar's chi-square test.

Table 3

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

		Controls			Controls	
NOS3 rs1799983		**G dom**	**T/T**		**T dom**	**G/G**
Cases	G dom *	167	13	T dom	41	57
	T/T	8	0	G/G	41	49
	Chi sq † = 0.762, *p* = 0.383			Chi sq = 2.296, *p* = 0.130		
		Controls			**Controls**	
NOS3 rs3918227		**A dom**	**C/C**		**C dom**	**A/A**
Cases	A dom	9	19	C dom	153	0
	C/C	24	101	A/A	0	0
	Chi sq = 0.372, *p* = 0.542			Chi sq = Not applicable		
		Controls			**Controls**	
GNB3 rs5442		**A dom**	**G/G**		**G dom**	**A/A**
Cases	A dom	2	16	G dom	179	0
	G/G	18	143	A/A	0	0
	Chi sq = 0.029, *p* = 0.864			Chi sq = Not applicable		
		Controls			**Controls**	
DDAH1 rs10158674		**C dom**	**T/T**		**T dom**	**C/C**
Cases	C dom	7	29	T dom	165	2
	T/T	28	107	C/C	4	0
	Chi sq = 0.0, *p* = 1.0			Chi sq = 0.167, *p* = 0.683		
		Controls			**Controls**	
DDAH1 rs233115		**A dom**	**G/G**		**G dom**	**A/A**

Cases	A dom		47	59	G dom		
			49	41	A/A		
	Chi sq = 0.750, $p = 0.386$				Chi sq = 2.531, $p = 0.112$		
						164	21
	G/G					11	0

* "G dom" refers to a recessive genetic model with the GG genotype compared with either GT or TT genotype.

† McNemar chi-square test.

Table 4

Univariate, logistic regression analysis of factors associated with preeclampsia.

Characteristic	*N* pairs	Model	OR	*p*-value
NOS3, rs1799983 (G allele)	188	Additive	0.870	0.496
		Dominant	1.704	0.353
		Recessive	1.376	0.202
NOS3, rs3918227 (A allele)	153	Additive	0.843	0.635
		Dominant	0.843	0.635
		Recessive	NApp	Napp
GNB3, rs5442 (A allele)	179	Additive	0.839	0.679
		Dominant	0.839	0.679
		Recessive	NApp	Napp
DDAH1, rs10158674 (C allele)	171	Additive	1.120	0.696
		Dominant	1.069	0.832
		Recessive	0.500	0.488
DDAH1, rs233115 (A allele)	196	Additive	1.006	0.976
		Dominant	1.208	0.428
		Recessive	1.804	0.204
Age at delivery (per year)	197		1.004	0.866
Nulliparity (yes)	197		3.269	<0.001
Gestation at first prenatal visit (per week from LMP)	175		0.973	0.144
Weight at first prenatal (per pound)	189		1.011	<0.001
Body mass index (per unit kg/m^2)	185		1.065	<0.001
Birth weight of infant (per gram)	171		0.999	<0.001
Mother's educational attainment (per year)	175		0.945	0.378
Maternal smoking during pregnancy (yes)	174		0.710	0.195
Gestational diabetes in current pregnancy (yes)	197		2.190	0.058

Table 5

Multivariate, conditional logistic regression analysis of factors associated with preeclampsia.

	OR	*p*-value
MODEL 1, all of following *		
Age at delivery	1.0823	0.0185
Nulliparous	6.8628	<0.001
BMI	1.0951	<0.001
MODEL 2, variables from Model 1 plus each of the following individually: †		
NOS3, rs1799983 (G allele recess)	1.4087	0.2354
NOS3, rs3918227 (A allele dom)	0.7356	0.4611
GNB3, rs5442 (A allele dom)	0.9147	0.8655
DDAH1, rs10158674 (C allele recess)	1.0165	0.9898
DDAH1, rs233115 (A allele recess)	2.2227	0.1578

*Covariates showing univariate significance, see Table 4.

†Genetic models are those with most significant p-values for that SNP, see Table 4.