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# GENETIC VARIANTS, IMMUNE FUNCTION AND RISK OF PRE-ECLAMPSIA 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 immune function and determine if they are associated with pre-eclampsia.

**Methods—**In a study of 66 cases and 130 matched controls, six single nucleotide polymorphisms (SNP) with either previously demonstrated or postulated modulating effects on the immune system were genotyped. Allele frequencies and various genetic models were evaluated by conditional logistic regression in both univariate and multiply adjusted models.

**Results—**Although most genetic variants lacked evidence of association with pre-eclampsia, the minor allele of the *CRP* related, rs1205 SNP in a dominant model with adjustment for age at delivery, nulliparity and body mass index, exhibited an odds ratio of 0.259 (95% CI of 0.08 – 0.81, p=0.020) in relation to severe pre-eclampsia (48 cases). The allelic prevalence of this variant was 46.1% in this population.

**Conclusion—**Of the six SNPs related to immune function in this study, a functional variant in the 3'UTR of the *CRP* gene was shown to be associated with severe pre-eclampsia in an American Indian population.

### Keywords

Pre-eclampsia; genetics; immune function; American Indian

## INTRODUCTION

Pre-eclampsia is a hypertensive condition unique to pregnancy characterized by new onset hypertension and proteinuria after 20 weeks of gestation (1, 2). Placental ischemia is central to the etiology and contributes to the imbalance of circulating angiogenic factors and endothelial dysfunction that underlie the clinical manifestations of pre-eclampsia (3, 4). Risk factors for pre-eclampsia (PE) include family history of both hypertension and pre-eclampsia (5–7) suggesting a genetic predisposition. The future risk for hypertension as a sequellae of pre-eclampsia for both mothers and their children provide further evidence for the influence of genetics (8–10).

Investigation of the familial predisposition to pre-eclampsia (PE) (11) has targeted gene candidates and specific genetic variants with postulated involvement in the pathophysiology of PE (12, 13); however, results of candidate single nucleotide polymorphisms (SNPs) studies have been inconsistent (14–16). Genetic variants associated with maladaptation of

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the maternal immune response mediated by cytokines and components of the innate immune system have been targeted in recent investigations of genetic markers for detection of PE risk and severity involving primarily Caucasian populations. In early pregnancy, Founds et al. (2009) reported evidence of dysregulation of genes associated with immune regulation and inflammation detected in chorionic villus sampling (CVS) tissues (17). There was no association with toll-like receptor 4 (TLR4) SNPs among Caucasian women with PE (18). A mutation of the human leukocyte antigen G (HLA-G) gene was associated with a severe form of PE, posited to underlie the reduced placental HLA-G protein expression found in patients with PE (19). However, in studies involving HLA-G SNPs among Norwegian and Brazilian women, there was no significant association with PE (20, 21). De Lima and colleagues (2009) reported an absence of SNP prevalences in cytokine genes (TNF-alpha, IL-6, IL-10, IFN-gamma) between Brazilian women with PE and those with normotensive pregnancy (22). In an Australian/New Zealand familial cohort with a genetic linkage to pre-eclampsia, Johnson et al. (2009) identified SNPs in the endoplasmic reticulum aminopeptidase (ERAP) 1 and 2 genes, central to regulation of immune and inflammatory responses(23). As the influence of background genetic variants and environmental interactions has the potential to modify phenotype, we investigated the prevalence of genetic variants within modulators of the immune system and their possible association with PE in an American Indian community. Some of these genes have been previously investigated (*MBL2, IL1A, CTLA4*) and others have not (*CRP*).

## METHODS

Recruitment for this case and matched control study has been ongoing from 8/04 to 12/10. 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 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 proteinuria in the same period. Cases were verified as meeting diagnostic criteria for PE if both of the following were identified:

**1)** At least 3 blood pressure values above either 140 mmHg systolic or 90 mmHg 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 is required.

**2)** Proteinuria as indicated by a 24 hour excretion of >300mg, or at least two +1 dipstick measurements in the absence of prior proteinuria.

These criteria were chosen to be compatible with the NHLBI Working Group on Research on Hypertension during Pregnancy definition (24).

Throughout most of this study, controls were ascertained by contact of the first individual to deliver before and after the index case; and the case definition was more permissive, meeting criteria if any 2 of the above criteria or a clinical diagnosis of pre-eclampsia were present. If a potential control declined participation, the woman delivering during the next prior or subsequent day was contacted. *For this analysis,* the case definition was restricted

as noted above and controls were rematched in a blinded fashion to cases, if they had delivered within the same year. The medical records of all controls were abstracted in the same way as cases. It was verified that controls did not meet either of the above 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.

Template DNA was provided by capillary blood samples collected on “FTA Classic Cards” (Whatman Inc) paper for the majority of participants. Three 1.2mm diameter “pellets” were punched from the cards and processed according to the manufacturer's recommendations. Since January of 2009, template DNA has been collected and processed using salivary samples and the Oragene (DNA Genotek Inc) system.

Pre-designed “TaqMan” (Applied Biosystems Inc) genotyping assays and protocols were implemented for SNPs on a real-time, Mini-Opticon (Bio-Rad Laboratories Inc), 4 color thermocycler. Controls were identified for at least 2 of the three possible genotypes (and “blank” controls) for each SNP and included with each analysis. In the case of rs3093077 and rs1205, HapMap genotypes (25) provided heterozygous control genotypes for samples obtained from the Coriell Institute for Medical Research. Control material of consistent genotype (replicated a minimum of 10 times) for each genotype was run with each set of samples, except in the case of homozygous “T” allele, where control material could not be identified for rs1800451. Internal controls for the other 2 genotypes of rs1800451 gave consistent results however. Samples detecting the more infrequent genotypes were generally duplicated in a minimum of two assays. Genotyping was robust, with only 2 samples failing analysis in over 3 attempts all genotypes determined. The number of pairs analyzed varied by SNP since 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 66 cases, 48 (73%) met criteria for “severe pre-eclampsia” according to the American College of Obstetricians and Gynecologists' definition (26). Of those with severe pre-eclampsia, 44 (91%) had at least 2 blood pressure measurements over 160 systolic or 110 diastolic, 16 (33%) had 3+ proteinuria by dipstick or over 5 gm per 24 hour collection, and 12 individuals met both criteria.

Table 1 summarizes the SNPs tested, population prevalences and consistency with Hardy-Weinberg equilibrium among cases and controls combined. The three *CRP* polymorphisms have been associated with increased hsCRP levels, both individually and as a haplotype group (27–29). The *IL1A*, rs3783550 C allele and pre-eclampsia were associated with an

odds ratio of 1.60;(27) and the *CTLA4*, rs231775 G allele with an odds ratio of 1.44 (27). The rs1800451 SNP in the *MBL2* gene is known to reduce the level of MBL protein expression; but the association of either genotype or serum levels with pre-eclampsia has been inconsistent (27, 29, 30).

Pertinent characteristics of the cases and controls are summarized in table 2. The prevalence of SNPs in genes encoding *CRP*, *IL1A*, *CTLA4*, and *MBL2* were not significantly different in cases versus controls. Significant differences between cases and controls were noted for nulliparity, weeks of gestation at delivery, body mass index (BMI), weight at first prenatal visit, and both systolic and diastolic blood pressure. Differences in near term delivery, birth weight of infants and blood pressures were not included in further models and felt likely to be consequences of PE or the applied diagnostic criteria, rather than etiologic.

Table 3 shows the genotypic results of paired cases and controls. McNemar chi-square analysis of pair wise comparisons (majority allele dominant, minor allele dominant) fails to demonstrate any significant associations.

Univariate conditional logistic regression results are shown in table 4 and confirm frequently reported associations between nulliparous status, maternal obesity and infant birth weight (33,34). Gestational diabetes also showed a significant association with pre-eclampsia in univariate analysis; but lacked significance (p=0.525) when included in a multivariate model with age at delivery, nulliparity and BMI. Models utilizing multivariate conditional logistic regression (table 5) continued to show robust, independent effects of nulliparity and obesity. Since age is clearly related to nulliparity, inclusion in multivariate models was felt necessary.

Analysis of the 48 cases meeting the definition of severe pre-eclampsia and their 104 matched controls did not change any of the previously mentioned univariate relationships with clinical factors; but did show a significant association with CRP-B, rs1205 (OR 0.354, p=0.025, 95%CI 0.143 – 0.877). Multivariate conditional logistic regression results continued to show a strong independent association between nulliparity and obesity. With multivariate adjustment, the CRP-B variant continued to show a significant association with severe preeclampsia, i.e. an odds ratio of 0.259 (95% CI of 0.08 – 0.81, p=0.020).

## DISCUSSION

The heritable transmission of risk for pre-eclampsia within families clearly points to a genetic etiology, though the identification of susceptibility genes has been elusive (11, 14–16, 31–33). The identification of diagnostic markers and therapeutic targets is hindered in the absence of a clear genetic association in the complex condition of pre-eclampsia. In this study, we investigated SNPs within genes associated with immune function as putative targets for the development of pre-eclampsia among American Indian women from the Turtle Mountain Band of Chippewa.

*CRP* genetic variants have been studied in relation to serum *CRP* concentrations and risk of coronary artery disease (34, 35), myocardial infarction (36), stroke (37, 38), hypertension (39), atherosclerosis (40), arterial pulse wave velocity (41, 42), and diabetes (28) however, to our knowledge, this is the first investigation of possible association between *CRP* genetic variants and pre-eclampsia. The *CRP* gene variants included in this study, in addition to rs1205, account for approximately 98% of the genetic variance in CRP serum levels among populations of European descent (34). However, the effect of genetic variation on CRP levels within the American Indian population is unknown. In this study, we reported a significant association between severe pre-eclampsia and the *CRP-B* SNP (rs1205), providing additional evidence for the significance of CRP in pre-eclampsia and novel

evidence for the genetic association of *CRP* with pre-eclampsia among American Indian women. The prevalence of the *CRP_A* and *CRP_C* SNPs were not significantly different by McNemar Chi square testing in cases as compared to controls. Univariate and multivariate conditional logistic regression analysis also indicated a lack of association of *CRP_A* and *CRP_C* with pre-eclampsia, as either typically defined, or severe pre-eclampsia.

Mannose-binding lectin (MBL) is proinflammatory, promoting maintenance of pregnancy and an inflammatory uterine environment. High levels of circulating maternal MBL have been associated with pre-eclampsia (43, 44), with levels thought to be influenced by *MBL2* polymorphisms. Although one report (45) noted a significantly higher prevalence of either the *MBL2* variant reported here, or another variant predisposing to low levels of MBL, the lack of association found in this study is consistent with other findings (46). Interleukin 1A (*IL1A*) SNPs have also been investigated as genetic candidates in the etiology of pre-eclampsia. Our finding of an absence of association between the *IL1A* rs3783550 SNP and pre-eclampsia after multivariate logistic regression analysis is similar to others, after adjusting for multiple testing (30). Cytotoxic T-lymphocyte-associated antigen-4 (*CTLA4*) rs231775 SNP was not associated with pre-eclampsia in a Brazilian population (47), also consistent with our findings in an American Indian population; but dissimilar to the findings in a Finnish population (48).

Our study findings indicate that well known risk factors such as nulliparity and maternal weight that are associated with pre-eclampsia risk in other population are significant in this American Indian community as well. Consistent with expected sequelae of pre-eclampsia, the factors of near term gestation at delivery, low birth weight and blood pressure were significantly different in the cases compared to controls.

Limitations of this study include the relatively small number of participants, which reduces the power available to detect associations; although this is counter-balanced to some extent by the abundant evidence linking the expression of CRP with various cardiovascular conditions with related pathophysiology. Strengths of this study include a well-defined phenotype of pre-eclampsia and the study design which employed an unbiased ascertainment of cases and controls. We present novel findings that a previously untested SNP is associated with severe pre-eclampsia in this American Indian community, providing essential new knowledge regarding allelic prevalence of critical importance to health care providers and planners in 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) |
|---|---|---|---|---|---|
| *CRP_A* | rs3093077 | T/G* | G = 5.2% | 2.8 – 7.6 | 0.374 |
| *CRP_B* | rs1205 | G/A, 3' UTR | A = 46.1% | 40.7 – 51.4 | 0.695 |
| *CRP_C* | rs1130864 | C/T, 3' UTR | C = 23.1% | 18.6 – 27.6 | 0.665 |
| *MBL2* | rs1800451 | C/T, G57E | T = 2.8% | 1.2 – 4.5 | 0.684 |
| *IL1A* | rs 3783550 | G/T, intronic | G = 45.0% | 39.8 – 50.3 | 0.682 |
| *CTLA4* | rs231775 | A/G, T17A | G = 49.1% | 43.9 – 54.4 | 0.702 |

*Transversion substitution (ABI), no info per NCBI

## Table 2

Characteristics of matched cases and controls.

| Characteristic | Cases | Controls | p value |
|---|---|---|---|
| Number (N) | 66 | 130 | N/A |
| CRP_A, rs3093077, G allele freq | 5/110 = 0.045 | 12/218=0.055 | 0.915* |
| CRP_B, rs1205, A allele freq | 46/108 = 0.426 | 107/224 = 0.478 | 0.442 |
| CRP_C, rs1130864, C allele freq | 28/110 = 0.256 | 50/228 = 0.219 | 0.560 |
| MBL2, rs1800451, T allele freq | 5/132 = 0.038 | 6/254 = 0.024 | 0.634 |
| IL1A, rs 3783550, G allele freq | 51/112 = 0.455 | 103/230 = 0.448 | 0.988 |
| CTLA4, rs231775, G allele freq | 58/120 = 0.483 | 114/230 = 0.496 | 0.915 |
| Age, mean years (SD) | 23.21 (5.63) | 23.74 (5.01) | 0.422** |
| Parity (N, % nulliparous) | 38 (57.6%) | 49 (37.7%) | <.001*** |
| Gestation at first prenatal visit mean wks from LMP, (SD) | 13.2 (7.5) | 13.8 (8.2) | 0.509 |
| Weight at first prenatal | 181.27 (44.96) | 157.28 (34.81) | <.001 |
| Body-Mass index (BMI) | 30.61 (6.90) | 26.99 (6.16) | <.001 |
| Gestational diabetes, N (% yes) | 11 (16.7%) | 6 (4.6%) | 0.081 |
| Weeks of gestation at delivery | 36.50 (3.93) | 39.24 (2.13) | <.001 |
| Birth weight of infant, grams | 3031 (1030) | 3393 (601) | 0.003 |
| Mother's educational attainment (years of education) | 11.87 (1.85) | 12.17 (2.17) | 0.297 |
| Maternal smoking, N (% yes) | 24/54 (44.4%) | 55/110 (50.0%) | 0.470 |
| Maternal smoking (Mean cigarettes smoked) | 5.36 (7.09) | 6.19 (11.74) | 0.523 |
| Mean systolic blood pressure | 165.2 (16.4) | 127.4 (10.6) | <.001 |
| Mean diastolic blood pressure | 97.8 (9.8) | 75.2 (7.5) | <.001 |

* Differences in allele frequency evaluated with Chi square test

** Differences between means evaluated with paired t test

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

*Am J Reprod Immunol*. Author manuscript; available in PMC 2013 February 1.

**Table 3**

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

| ***CRP_A* rs3093077** | | **Controls** | | | **Controls** | |
|---|---|---|---|---|---|---|
| | | **T dom** | **G/G** | | **G dom** | **T/T** |
| Cases | T dom | 104 | None | G dom | 2 | 7 |
| | G/G | None | None | T/T | 9 | 86 |
| | Not applicable | | | Chi sq =0.06, p=0.803 | | |

| ***CRP_B* rs1205** | | **Controls** | | | **Controls** | |
|---|---|---|---|---|---|---|
| | | **G dom** | **A/A** | | **A dom** | **G/G** |
| Cases | G dom | 63 | 16 | Adom | 54 | 16 |
| | A/A | 19 | 6 | G/G | 27 | 7 |
| | Chi sq =0.11, p=0.735 | | | Chi sq =2.33, p=0.127 | | |

| ***CRP_C* rs1130864** | | **Controls** | | | **Controls** | |
|---|---|---|---|---|---|---|
| | | **C dom** | **T/T** | | **T dom** | **C/C** |
| Cases | C dom | 18 | 22 | T dom | 95 | 5 |
| | T/T | 24 | 43 | C/C | 7 | 0 |
| | Chi sq =0.02, p=0.883 | | | Chi sq =0.08, p=0.773 | | |

| ***MBL2* rs10158674** | | **Controls** | | | **Controls** | |
|---|---|---|---|---|---|---|
| | | **C dom** | **T/T** | | **T dom** | **C/C** |
| Cases | C dom | 127 | 0 | T dom | 0 | 7 |
| | T/T | 0 | 0 | C/C | 6 | 114 |
| | Not applicable | | | Chi sq =0.00, p=1.000 | | |

| ***IL1A* rs 3783550** | | **Controls** | | | **Controls** | |
|---|---|---|---|---|---|---|
| | | **G dom** | **T/T** | | **T dom** | **G/G** |
| Cases | G dom | 56 | 23 | T dom | 77 | 22 |
| | T/T | 20 | 15 | G/G | 12 | 3 |
| | Chi sq =0.09, p=0.760 | | | Chi sq =2.38, p=0.123 | | |

| *CTLA4* rs231775 | | Controls | | | Controls | |
|---|---|---|---|---|---|---|
| | | Adom | G/G | | G dom | A/A |
| Cases | Adom | 70 | 22 | G dom | 64 | 21 |
| | G/G | 17 | 5 | A/A | 20 | 9 |
| | Chi sq =0.41, p=0.522 | | | Chi sq =0.00, p=1.000 | | |

* "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 pre-eclampsia.

| Characteristic | N pairs | Model | OR | p Value |
|---|---|---|---|---|
| *CRP_A*, rs3093077, (T allele) | 105 | Additive | 1.267 | 0.686 |
| | | Dominant | Not Applic | |
| | | Recessive | 0.833 | 0.770 |
| *CRP_B*, rs1205, (G allele) | 105 | Additive | 1.159 | 0.580 |
| | | Dominant | 0.845 | 0.716 |
| | | Recessive | 0.648 | 0.264 |
| *CRP_C*, rs1130864, (C allele) | 108 | Additive | 1.098 | 0.756 |
| | | Dominant | 0.972 | 0.938 |
| | | Recessive | 0.541 | 0.370 |
| *MBL2*, rs1800451, (C allele) | 113 | Additive | 0.592 | 0.420 |
| | | Dominant | Not Applic | |
| | | Recessive | 1.688 | 0.421 |
| *IL1A*, rs 3783550, (G allele) | 115 | Additive | 0.949 | 0.838 |
| | | Dominant | 1.146 | 0.748 |
| | | Recessive | 1.329 | 0.515 |
| *CTLA4*, rs231775, (A allele) | 114 | Additive | 1.047 | 0.859 |
| | | Dominant | 1.205 | 0.641 |
| | | Recessive | 1.101 | 0.828 |
| Age at delivery (per year) | 128 | | 0.977 | 0.448 |
| Nulliparity (yes) | 130 | | 2.745 | 0.004 |
| Gestation at first prenatal visit (per week from LMP) | 118 | | 0.979 | 0.333 |
| Weight at first prenatal (per pound) | 128 | | 1.012 | 0.003 |
| Body-Mass index (per unit Kg/meter$^2$) | 122 | | 1.086 | 0.002 |
| Birth weight of infant (per gram) | 112 | | 0.999 | 0.036 |
| Mother's educational attainment (per year) | 112 | | 0.906 | 0.238 |
| Maternal smoking (mean cigarettes smoked per day) | 110 | | 0.985 | 0.184 |
| Gestational diabetes in current pregnancy (yes) | 130 | | 2.780 | 0.049 |

## Table 5

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

| | Pre-eclampsia | | Severe Pre-eclampsia | |
|---|---|---|---|---|
| *MODEL 1, all of following* * | | | | |
| | OR | P value | OR | P value |
| Age at delivery | 1.036 | 0.398 | 1.027 | 0.586 |
| Nulliparous | 4.274 | 0.003 | 4.520 | 0.009 |
| BMI | 1.093 | 0.002 | 1.094 | 0.007 |
| *MODEL 2, Age, nulliparity and BMI, plus each of the following individually*:** | | | | |
| *CRP_A*, rs3093077, (T allele additive) | 1.201 | 0.799 | 0.637 | 0.589 |
| *CRP_B*, rs1205, (A allele dom) | 0.555 | 0.197 | 0.259 | 0.020 |
| *CRP_C*, rs1130864, (T allele dom) | 0.435 | 0.305 | 0.418 | 0.293 |
| *MBL2*, rs1800451, (T allele dom) | 1.533 | 0.599 | 1.297 | 0.775 |
| *IL1A*, rs 3783550, (T allele dom) | 1.321 | 0.530 | 1.618 | 0.410 |
| *CTLA4*, rs231775, (A allele dom) | 1.409 | 0.455 | 1.309 | 0.620 |

* Covariates showing univariate significance, plus age, which interacts with nulliparity.

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