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# Associating Symptom Phenotype and Genotype in Preeclampsia

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**Abstract**

Preeclampsia is a complex genetic disorder with an incompletely understood pathogenesis. Its phenotype may be better elucidated by integrating symptoms. This study aimed to identify symptoms by gestational age and associations with novel preeclampsia candidate genes. Women with a history of preeclampsia recruited from The Preeclampsia Registry completed clinical/demographic, symptom surveys and provided medical records. DNA extracted from saliva was processed with multiplexed assays for eight single-nucleotide polymorphisms (SNPs) selected to tag candidate genes and/or located in symptom susceptibility regions. Groups with versus without symptoms were compared using $\chi^2$. Associations between SNPs and symptoms were analyzed as genotype categories and presence/absence of the variant allele. Logistic regression modeling was conducted with exploratory $p = .05$. In 114 participants, 113 reported at least 1 of the 18 symptoms. Symptoms varied by trimester. Nine symptoms were associated with seven SNPs. Visual disturbances were associated with three SNPs and nausea/vomiting with two SNPs. Modeling adjustment for maternal age and parity resulted in 15 associations between 9 symptoms and 8 SNPs. Medical records demonstrated 100% concordance with self-reported diagnosis and 48% concordance with reported severity. Findings indicated novel symptom–genotype associations in preeclampsia. The small sample was self-selected, but results support future studies including medical records review. When validated, these results may lead to holistic phenotyping of women to characterize subsets of preeclampsia. This approach may optimize health in pregnancy and later life for mothers and offspring through prediction, prevention, and precision nursing care.

**Keywords**

symptom phenotype, candidate SNP, candidate gene, prediction, prevention, precision health, systems biology

**Gene names and symbols:** *CACNA1A* = Ca2+ channel 1-subunit gene; *CFH* = complement factor H; *ELL2* = elongation factor for RNA polymerase II 2; *F11R* = F11 receptor; FN1= fibronectin 1; FSTL3 = follistatin like 3; S100A8 = S100 calcium binding protein A8; *SEMA3C* = semaphorin 3C.

Preeclampsia complicates 3–8% of all pregnancies (Lisonkova & Joseph, 2013; Miller et al., 2017). Hypertensive disorders of pregnancy, the second leading cause of maternal mortality worldwide, account for 14% of all maternal deaths (Say et al., 2014). Preeclampsia is defined by the new onset of hypertension and proteinuria after 20 weeks of gestation; however, if proteinuria does not occur, diagnosis is made in the presence of hypertension with thrombocytopenia, renal insufficiency, impaired liver function, cerebral or visual changes, or pulmonary edema (American College of Obstetricians and Gynecologists [ACOG], 2013). These pathologies can lead to sudden life-threatening events—placental abruption, disseminated coagulopathy, renal failure, respiratory failure, ruptured liver capsule, and eclampsia (Sibai, Dekker, & Kupferminc, 2005). Mothers and newborns who survive these severe consequences of preeclampsia are at increased risk of later-life cardiovascular disease (Kajantie, Eriksson, Osmond, Thornburg, & Barker, 2009; Mosca et al., 2011).

Prevention and precision care of preeclampsia are hindered by the lack of valid, robust early predictive biomarkers (ACOG, 2013; Myatt et al., 2013), leaving current nursing, midwifery, and obstetrical management to rely on detection of signs and symptoms for clinical diagnosis. The molecular pathogenesis of the disease remains incompletely understood, but familial clustering indicates a genetic basis, with

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heritability estimates of 25–54% in populations (Founds, Dorman, & Conley, 2008; Salonen Ros, Lichtenstein, Lipworth, & Cnattingius, 2000). Preeclampsia is a complex genetic disorder with many genes, environmental factors, and interactions among the two that have not yet been delineated, in part because subtype or subsets of the phenotype remain to be better characterized (Leavey et al., 2016).

Abnormal placental development has been identified as a root cause of preeclampsia, such that delivery is considered to be the only known cure (Amaral, Wallace, Owens, & LaMarca, 2017). Genetic and immunologic factors interacting with behavioral and environmental factors predispose the maternal–fetal system to impaired trophoblast invasion, which results in shallow placentation and incomplete physiologic conversion of the maternal spiral arteries (Brosens, Pijnenborg, Vercruysse, & Romero, 2011; Ilekis et al., 2016). The unchanged spiral arteries retain vasoreactivity that induces vasoconstriction, placental hypoperfusion, and associated systemic dysfunction such as increased inflammatory activation, endothelial dysfunction, and oxidative stress (Roberts et al., 1989; Roberts & Hubel, 2004). Villus growth restricted by overcrowding near term may cause hypoperfusion leading to late-onset preeclampsia (Redman, Sargent, & Staff, 2014).

In an effort to discover early molecular biomarkers of an aberration in placental development occurring before the onset of clinical signs and symptoms of preeclampsia, Founds and colleagues (2009) conducted a global gene-expression microarray analysis in first trimester tissues of preeclampsia origin. Of 157 women undergoing chorionic villus sampling (CVS) for advanced maternal age who donated surplus tissue, 4 developed preeclampsia (2.5%). Researchers matched these cases, at ~11.5 weeks of gestation, with eight controls and found that 36 candidate genes were differentially expressed in pathways relevant to maternal-fetoplacental development such as those related to immune/inflammation, invasion, and migration in early preeclampsia placentas. Quantitative real-time polymerase chain reaction analyses supported the novel panel of 36 candidates and demonstrated large effect sizes in downregulated expression of *FSTL3* and *LAIR2* early in preeclampsia (Founds et al., 2011). Among the panel of CVS candidate genes, nearly 40% were positional candidates located in preeclampsia linkage regions (Founds, 2011). Further preliminary studies demonstrated genetic and epigenetic differences in case versus control maternal-fetoplacental dyads for 25 single-nucleotide polymorphisms (SNPs) in *ELL2*, *F11R*, *FN1*, and *SEMA3C* and in 20 CpG sites in *ELL2* and *F11R* (Founds et al., 2012). These findings in samples that were not part of the original CVS microarray analysis provided additional independent verification of the novel preeclampsia candidate genes.

From a systems biology conceptual framework in which the whole is greater than the sum of its parts, subsets or different types of preeclampsia may be better elucidated by integrating women's symptom experiences with biophysical and behavioral factors to phenotype this disorder across gestation (Founds, 2009; Founds, 2017). Although symptoms have been considered to be nonspecific for the diagnosis of preeclampsia or its extremely severe form, hemolysis, elevated liver enzymes, or low platelets (HELLP) syndrome, clinicians at prenatal visits routinely inquire about persistent headache, visual disturbance, and epigastric or right upper abdominal pain as hallmark "warning signs" of abnormality (ACOG, 2013). Known symptomatology stems from historic anecdotal medical practice substantiated by retrospective descriptive data and the Delphi method such as percentages of women with the particular symptom at admission for HELLP syndrome and eclampsia (Thangaratinam et al., 2011). These subjective symptoms are prodromal within 1 week preceding the severe and potentially lethal pathology of preeclampsia, when it is already too late for prevention (Black, 2007; Steegers, von Dadelszen, Duvekot, & Pijnenborg, 2010). A meta-analysis evaluating the prediction of preeclampsia by symptoms indicated that the presence of symptoms is more predictive of complications in preeclampsia than the absence of symptoms is in excluding adverse events (Thangaratinam et al., 2011).

In a few nursing studies, researchers focused on the classical late-stage symptoms in worsening antepartum disease (Black, 2007; Kidner & Flanders-Stepans, 2004) and postpartum preeclampsia (Atterbury, Groome, Hoff, & Yarnell, 1998). Using qualitative methods, investigators shed light on underrecognized antenatal symptoms. Malaise, vertigo, and an inability to concentrate were added to a symptom inventory through interviews with women newly diagnosed with gestational hypertension or preeclampsia (Black, 2007). Back pain, excessive fatigue, shortness of breath, and not feeling right were identified by women with HELLP syndrome (Kidner & Flanders-Stepans, 2004).

To date, there is no genotype data for symptoms in preeclampsia; however, examples of symptom–genotype studies in other women's health issues support the feasibility of this direction for advancing preeclampsia research. Increased susceptibility to symptoms of postpartum mood, operationalized as severe emotional problems or severe depression in the time period following pregnancy, was associated through genome-wide study with variations in chromosome regions 1q21.3-q32.1 and 9p24.3-p22.3 (Mahon et al., 2009). Genotype difference in *IL16* was more evident in women with endometriosis who experienced pain symptoms ($p < .001$) than in those without pain when compared with controls (Gan, Lin, Zhang, Yu, & Hu, 2010). Although researchers in another study found that women with a history of migraine headaches prior to pregnancy had a 3.5-fold increased risk of preeclampsia, no genotype data were generated in this study (Sanchez, Qiu, Williams, Lam, & Sorensen, 2008). The investigators suggested, however, that a gene for migraine susceptibility on chromosome 19p13 might also be associated with preeclampsia based on others' finding that familial migraines were associated with this susceptibility region in which specific missense mutations in *CACNA1A* were identified (Ophoff et al., 1996).

The aforementioned studies taken together suggest the plausibility of associating women's symptom experience in pregnancy with variability in these chromosomal regions. Interestingly, 5 of the 36 discovery-based preeclampsia

candidate genes (Founds et al., 2009) are positional candidates located in susceptibility regions previously associated by population linkage studies to symptoms. Preeclampsia candidates *CFH* in 1q32, *F11R* in 1q21.2-q21.3, *S100A8* in 1q21, and CDNA: FLJ22732 fis clone HSI15880 in 1q31.3 occur at loci in the 1q21.3-q32.1 region, which was associated with postpartum mood susceptibility (Mahon et al., 2009), and preeclampsia candidate *FSTL3* in 19p13.3 is located in the 19p13 susceptibility region for severe headaches (Ophoff et al., 1996). Rather than assuming that polymorphisms in the CVS preeclampsia candidate genes would associate specifically with mood or migraines, we focused on the potential for association with subjective symptom phenotype in the current investigation.

Little is known about the symptoms women who subsequently develop preeclampsia experience prior to diagnosis or early in pregnancies before deterioration to the late and potentially lethal complications or whether symptom phenotype associates with preeclampsia genotype. These gaps in knowledge led us to hypothesize that polymorphisms in some of the CVS candidate genes may be associated with symptom phenotype in preeclampsia. In the present study, we aimed to identify women's symptoms across gestation in preeclampsia pregnancies and to explore associations of these symptoms with a subset of the novel preeclampsia candidate genes (Founds et al., 2009).

## Method

This pilot candidate gene association study was developed in collaboration with the Preeclampsia Foundation executive director (E.T.). Both the principal investigator's (PI) institution and the Foundation provided institutional review board approval. The Foundation was established as a nonprofit advocacy organization in 2000. Its mission is to reduce maternal and infant illness and death due to preeclampsia, HELLP syndrome, and other hypertensive disorders of pregnancy by providing patient support and education, raising public awareness, catalyzing research, and improving health-care practices. Approximately 4,500 unique visitors access the Foundation's website daily. The Community Forum consists of 19,599 members who have participated over the course of several years.

### *Sample*

Women with a history of preeclampsia were recruited from The Preeclampsia Registry (TPR; http://www.preeclampsiaregistry.org/). At the time of this study, there were 1,660 participants consented to participate in TPR. Women with symptoms of problems during pregnancy may rationalize them away, turn to significant others for interpretation, and experience stress with complications (Stringer, Gennaro, Deatrick, & Founds, 2008); therefore, in this pilot, we recruited women who previously birthed and had a history of preeclampsia to obtain preliminary data to examine the feasibility of future studies with a pregnant population. Women were eligible to participate if they were over 18 years of age and had survived a preeclampsia pregnancy 5 years or less prior to participating in this study. Exclusion criteria included gestational diabetes or preexisting hypertension before the pregnancy in which preeclampsia was diagnosed. We planned for a sample size of 175 to produce a two-sided 95% confidence interval (CI) with a distance from the mean to the limits that is equal to 0.149 when the estimated standard deviation is assumed as 1.000 and a width equal to 0.148, assuming a baseline proportion of 0.5 when estimating population group proportions. A $\chi^2$ power analysis (for SNP variant present/absent vs. symptom present/absent) revealed that the proposed sample size of 175 has 80% power to detect a small effect size of 0.2 using 1 degree of freedom with a significance level of $\alpha$ at .05.

### *Procedures*

We distributed and collected study instruments and other data with informed consent through the Foundation's secured electronic TPR. In order to identify women's symptoms in preeclampsia pregnancies and associations with novel preeclampsia candidate genes, we conducted the following procedures: (1) asked participating women to complete a survey of clinical and demographic factors and preeclampsia symptoms, (2) accrued and abstracted medical records, (3) collected DNA specimens, and (4) identified genotype associations between SNPs in two groups of women with versus without symptoms in preeclampsia pregnancies. Participants provided consent for the collection of medical records. TPR staff deidentified medical records, self-reported data, and DNA samples before studying them.

### *Survey Items and Medical Records*

Participants reported their clinical and demographic data in TPR. Recall bias thus posed a potential limitation for this study. Prior reports of fairly accurate recall of preeclampsia pregnancies, specifically, 0.87 sensitivity and 0.98 specificity of maternal self-report (Coolman et al., 2010), along with missing data in self-reports of gestational age for pregnancy events (Yawn, Suman, & Jacobsen, 1998) led to our plan to abstract medical records data in this study, which would provide clinical confirmation for the diagnosis of preeclampsia (Stuart et al., 2013). Participants submitted the medical record documents through attachment in the TPR database, and TPR redacted them before providing the records to the PI. The investigators abstracted diagnoses from the medical records according to criteria of the ACOG Task Force on Hypertension in Pregnancy (2013).

A symptom survey that had been previously deployed in TPR was augmented with research literature-based data to total 49 items covering 20 symptoms, some with additional descriptor items such as severity, location, and quality of the symptom. Each item required a yes/no response to presence of the symptom or symptom feature. The item "other" provided space for the participant to comment on any symptom that had not been included in the survey. The complete symptom checklist did not have established psychometric properties; however, the

survey included 11 symptom items that prior research had demonstrated to produce a content validity index of .93 and Cronbach's α of .77 (Black, 2007; Black & Morin, 2014). The original TPR survey listed time intervals during which each symptom occurred, that is, three trimesters, labor, birth, postpartum intervals, and "I don't know." One time period could be selected per symptom. It was infeasible for this pilot study to reprogram the expanded survey with weekly interval options across gestation; therefore, we utilized the TPR time categories with the checklist.

## DNA Samples

Samples of participants' DNA, which was extracted from saliva and passed quantitation and quality assurance checks (Picogreen and Nanodrop, ThermoFisher, Pittsburgh, PA), were provided through TPR for use in this study.

## Genotype Data Collection

We genotyped SNPs in the preeclampsia candidate genes identified by the previous global gene expression study (Founds et al., 2009; Founds et al., 2011). Selection of SNPs was prioritized on tagging candidate genes (tagging SNPs [tSNPs]) and/or biologic plausibility based on position in symptom susceptibility loci (Table 1). HapMap build 36 Phase 3 Draft 2 was available at the time of SNP selection. Tagger pairwise selection with RS square cutoff .8 and minor allele frequency (MAF) in Caucasian ancestry (CEU) with .2 cutoff were used to identify the candidate SNPs. The SNP in S100A8 had an MAF below cutoff because few SNPs in this positional candidate were available at that time (Table 1). CEU was consistent with the CVS microarray specimen donors (Founds et al., 2009). Eight TaqMan Predesigned SNP Genotyping Assays (Applied Biosystems, Foster City, CA) in multiplexed pools were utilized with Tecan Rearray according to standardized protocols by the University Genomic Research Core.

## Data Analysis

Detailed descriptive analyses of the data were conducted using standard descriptive summaries (i.e., means, standard deviations, percentiles, and ranges) and graphical techniques (i.e., histograms and scatter plots). Each SNP was examined by allele, genotype, and Hardy–Weinberg equilibrium (HWE). The exact test was implemented in PLINK software (v1.07) to examine HWE ($p > .05$) in all SNPs genotyped (Purcell et al., 2007). The associations between candidate gene SNPs and presence of preeclampsia symptoms were evaluated. Data were analyzed as both (a) genotype categories and (b) presence/absence of the variant allele, where the heterozygotes are combined with the variant homozygotes and compared to the homozygote wild type. The bivariate associations between each SNP and symptom were explored by using $\chi^2$ test and/or Fisher's exact test initially. The potentially important SNPs were selected for multivariate analysis. Then, multivariate logistic regression models were run to examine the relationships between SNPs, which were selected from univariate analysis, and symptoms while controlling for covariates. The odds ratio (*OR*), 95% CI, and *p* value were reported for each SNP. Given the exploratory nature of the study, the potential inflation of Type I error due to the screening of multiple SNPs was not adjusted for; however, the α for screening SNPs of interest was ≤.05. SAS 9.4 software (SAS Institute Inc., Cary, NC) was used for all the analyses.

**Table 1.** Candidate Single-Nucleotide Polymorphisms (SNPs).

| Preeclampsia Candidate Gene and Function | Locus | SNP and Function | Position | MAF |
|---|---|---|---|---|
| *CFH:* Complement activation, innate defense; binds to C3b, accelerates the decay of the alternative pathway convertase C3bBb, and acts as a cofactor for complement factor I to regulate the function of the alternative complement pathway in fluid phase and on cellular surfaces[a] | 1q32 | rs10922096[b] Intron variant | 194,929,082 | .469 |
| | | rs1329428[b] Intron variant | 194,969,433 | .424 |
| *F11R:* Regulator of tight junction assembly in epithelia[c] | 1q21.2–q21.3 | rs836 UTR variant 3 prime | 159,234,848 | .279 |
| | | rs11265544 Unknown | 159,272,643 | .395 |
| | | rs7546890 Unknown | 159,262,016 | .438 |
| *FSTL3:* Leukemogenesis, osteoclast differentiation, hematopoiesis; binds and regulates growth and differentiation factors during development[d] | 19p13.3 | rs1046253[b] UTR variant 3 prime | 634,221 | .230 |
| | | rs28364866[b] Intron variant | 632,193 | .155 |
| *S100A8:* Cell cycle progression and differentiation; inflammatory processes, immune response[e] | 1q21 | rs3795391 Intron variant | 153,390,629 | .088 |

*Note.* MAF = minor allele frequency; UTR = untranslated region. [a]http://www.genecards.org/cgi-bin/carddisp.pl?gene=CFH&keywords=CFH. [b]Tagging SNP. [c]http://www.genecards.org/cgi-bin/carddisp.pl?gene=F11R&keywords=F11R. [d]http://www.genecards.org/cgi-bin/carddisp.pl?gene=FSTL3&keywords=FSTL3. [e]http://www.genecards.org/cgi-bin/carddisp.pl?gene=S100A8&keywords=S100A8.

**Table 2.** Symptom Frequency by Trimester.

| | Reported Symptom During Gestation | Reported in First Trimester (Weeks 1–12) | Reported in Second Trimester (Weeks 13–27) | Reported in Third Trimester (Week 28–Birth) |
|---|---|---|---|---|
| Symptom | *n* (%) | *n* (%)[a] | *n* (%) | *n* (%) |
| Swelling | 93 (82.3) | 0 (0.00) | 33 (35.5) | 55 (59.1) |
| Fatigue/tiredness | 87 (77.0) | 47 (54.0) | 12 (13.8) | 27 (31.0) |
| Just not feeling right | 85 (79.4) | 5 (5.9) | 18 (21.2) | 54 (63.5) |
| Nausea and/or vomiting | 71 (62.8) | 48 (67.6) | 4 (5.6) | 18 (25.4) |
| Indigestion/heartburn | 69 (59.5) | 14 (20.3) | 31 (44.9) | 22 (31.9) |
| Headaches | 66 (58.9) | 10 (15.2) | 17 (25.8) | 34 (51.5) |
| Sleep difficulties | 52 (46.3) | 9 (17.3) | 16 (30.8) | 24 (46.2) |
| Abdominal pain | 49 (45.1) | 1 (2.0) | 9 (18.4) | 35 (71.4) |
| Back pain | 46 (41.4) | 5 (10.9) | 11 (23.9) | 29 (63.0) |
| Visual disturbances[b] | 42 (37.5) | 2 (4.8) | 11 (26.2) | 20 (47.6) |
| Shortness of breath | 39 (35.5) | 2 (5.1) | 13 (33.3) | 20 (51.3) |
| Low appetite | 36 (32.4) | 18 (50.0) | 5 (14.0) | 11 (31.0) |
| Other | 34 (34.2) | 6 (17.6) | 9 (26.5) | 16 (47.1) |
| Trouble thinking clearly/ altered consciousness | 30 (27.5) | 2 (6.7) | 11 (36.7) | 13 (43.3) |
| Vertigo/dizziness | 25 (22.7) | 4 (16.0) | 10 (40.0) | 8 (32.0) |
| Chest pain | 16 (14.6) | 0 (0.00) | 2 (12.5) | 12 (75.0) |
| Abnormal bleeding before delivery | 15 (13.4) | 13 (86.7) | 1 (6.7) | 1 (6.7) |
| Palpitations | 14 (12.2) | 2 (14.3) | 5 (35.7) | 7 (50.00) |

*Note.* $n = 113$ women who reported any symptom.
[a]Percentage provided for each trimester is the proportion of women who reported the specific symptom during that particular trimester out of all women who reported any symptom during pregnancy. Numbers by trimester may not total all women reporting the symptom if the timing during gestation was not identified.
[b]1 (0.02) woman reported "don't know."

## Results

We recruited 114 participants with a history of preeclampsia in pregnancy, who completed the surveys and provided DNA samples through TPR. Average age at the time of the affected pregnancy was 30 years (range = 19–56, $SD = \pm 4.9$). Most of the women (103 [90%]) were nulliparous in the affected pregnancy. The majority identified as Caucasian (95%) and was married or in a relationship (96%), while all had completed at least a high school education at the time of their preeclampsia pregnancy. Participants' prepregnancy body mass index averaged 25.7 kg/m$^2$ (range = 17.5–43.7, $SD = \pm 5.96$). They had given birth at an average of 34.1 weeks of gestation (range = 23–40 weeks, $SD = \pm 4.4$). Many had been diagnosed with severe forms of preeclampsia, with 44 (38.6%) having had early-onset preeclampsia (<34 weeks, 0 days) or HELLP syndrome. The group of 38 women (33%) diagnosed with HELLP were no different in symptoms or genotypes than women diagnosed with preeclampsia only.

Of the 114 participants, 29 (25%) submitted medical records. Chart review indicated that all of these participants (100%) had a diagnosis on record of a hypertensive disorder in the affected pregnancy. There was 48% concordance of the medical record diagnosis with self-reported type or severity of preeclampsia, eclampsia, or HELLP syndrome. For some participants, only partial records were collected during the funding period.

Nearly, all participants (113) reported at least 1 of the 18 symptoms (Table 2). Timing was the only data obtained for any descriptive feature item. Data were recategorized from 10 to 4 groups for tabulation. Swelling was the symptom participants reported most frequently, while palpitations were least frequently reported. Of the 18 symptoms, 12 increased in frequency of reporting each trimester across gestation, whereas fatigue/tiredness, nausea and/or vomiting, and low appetite were highest in the first trimester, decreased in the second trimester, but increased again in the third trimester. Two symptoms, indigestion/heartburn and vertigo/dizziness, were present in the first trimester, were highest in the second trimester, but decreased in the third trimester. Abnormal bleeding was the only symptom that was most frequent in the first trimester, but it was also reported once in each of the second and third trimesters. Other symptoms written in by the participants included physical signs and subjective experiences of anxiety, overwhelm, feeling less fetal movement, and pain in the right shoulder.

We found that seven SNPs were associated with nine symptoms in these preeclampsia pregnancies. All genotyped SNPs were in HWE. Visual disturbances and nausea and/or vomiting were associated with multiple SNPs (Table 3). The tSNP rs28364866 in *FSTL3* had no symptom association. Symptoms that were associated with both the genotype and the variant included headaches and visual disturbances with rs1329428, visual disturbances and fatigue/tiredness with rs3795391,

**Table 3.** Symptoms Associated With Genotype in Preeclampsia.

| | | | With Symptom | Without Symptom | |
|---|---|---|---|---|---|
| Symptom | Gene SNP | Genotype | *N* (%) | *N* (%) | *p* Value |
| Headaches | *CFH* rs1329428 | C/C | 30 (27.3) | 10 (9.1) | .02 |
| | | C/T | 24 (21.8) | 28 (25.5) | |
| | | T/T | 11 (10.0) | 7 (6.4) | |
| | *CFH* rs1329428 | C/C vs. C/T + T/T | 30 (27.3) | 10 (9.1) | .02 |
| | | | 35 (31.8) | 35 (31.8) | |
| Visual disturbances | *CFH* rs1329428 | C/C | 20 (18.2) | 19 (17.3) | .05 |
| | | C/T | 14 (12.7) | 39 (35.5) | |
| | | T/T | 7 (6.4) | 11 (10.0) | |
| | *CFH* rs1329428 | C/C vs. C/T + T/T | 20 (18.2) | 19 (17.3) | .04 |
| | | | 21 (19.1) | 50 (45.5) | |
| | *S100A8* rs3795391 | C/C | 0 (0.0) | 1 (0.9) | .002 |
| | | C/T | 1 (0.9) | 17 (16.0) | |
| | | T/T | 39 (36.8) | 48 (45.3) | |
| | *S100A8* rs3795391 | C/C + C/T vs. T/T | 1 (0.9) | 18 (17.0) | .001 |
| | | | 39 (36.8) | 48 (45.3) | |
| | *F11R* rs7546890 | C/C vs. C/T + T/T | 15 (13.8) | 12 (11.0) | .02 |
| | | | 25 (22.9) | 57 (52.3) | |
| Fatigue/tiredness | *S100A8* rs3795391 | C/C | 1 (0.9) | 0 (0.0) | .03 |
| | | C/T | 10 (9.3) | 8 (7.5) | |
| | | T/T | 73 (68.2) | 15 (14.0) | |
| | *S100A8* rs3795391 | C/C + C/T vs. T/T | 11 (10.3) | 8 (7.5) | .03 |
| | | | 73 (68.2) | 15 (14.0) | |
| Other | *S100A8* rs3795391 | C/C + C/T vs. T/T | 2 (1.9) | 16 (15.5) | .05 |
| | | | 30 (29.1) | 55 (53.4) | |
| Nausea and/or vomiting | *S100A8* rs3795391 | C/C | 1 (0.9) | 0 (0.0) | .03 |
| | | C/T | 7 (6.5) | 11 (10.3) | |
| | | T/T | 59 (55.1) | 29 (27.1) | |
| | *FSTL3* rs1046253 | A/A | 1 (0.9) | 4 (3.7) | .02 |
| | | G/A | 30 (27.8) | 9 (8.3) | |
| | | G/G | 36 (33.3) | 28 (25.9) | |
| Indigestion/heartburn | *F11R* rs11265544 | A/A | 22 (20.6) | 19 (17.8) | .04 |
| | | A/C | 32 (29.9) | 17 (15.9) | |
| | | C/C | 15 (14.0) | 2 (1.9) | |
| | *F11R* rs11265544 | A/A + A/C vs. C/C | 54 (50.5) | 36 (33.6) | .03 |
| | | | 15 (14.0) | 2 (1.9) | |
| Palpitations | *F11R* rs836 | A/A | 3 (2.8) | 6 (5.6) | .006 |
| | | G/A | 1 (0.9) | 43 (40.2) | |
| | | G/G | 9 (8.4) | 45 (42.1) | |
| Shortness of breath | *F11R* rs836 | A/A + A/G vs. G/G | 24 (22.0) | 29 (26.6) | .05 |
| | | | 15 (13.8) | 41 (37.6) | |
| Back pain | *CFH* rs10922096 | C/T + T/T vs. C/C | 28 (25.7) | 50 (45.9) | .05 |
| | | | 18 (16.5) | 13 (11.9) | |

*Note.* SNP = single-nucleotide polymorphism.

and indigestion/heartburn with rs11265544. Symptoms that were associated with the genotype but not the variant included nausea and/or vomiting with rs3795391 and rs1046253 and palpitations with rs836. Symptoms that were associated with the variant allele but not the genotype included visual disturbances with rs7546890, other with rs3795391, shortness of breath with rs836, and back pain with rs10922096.

Logistic regression adjusting for maternal age and parity (dichotomized as history of 1 vs. >1 birth) resulted in 15 associations between nine symptoms and eight SNPs in the preeclampsia candidate genes (Table 4). There was no effect of symptom timing on multivariate modeling of the SNP data. Most of the associations demonstrated lower odds of symptoms by SNPs. Both Headaches and visual disturbances had lower likelihood with C/T genotype and absence of C/C in rs1329428. Visual disturbances were also less likely in the absence of both T/T in rs3795391 and C/C rs7546890. Fatigue/tiredness and nausea and/or vomiting were less likely with T/T absent in rs3795391. Lower likelihood of palpitations was associated with the A/G genotype in rs836. Back pain and chest pain were less likely when the C/C genotype in rs10922096

**Table 4.** Odds of Symptoms by SNPs Adjusted for Maternal Age and Parity.

| Symptom | Gene SNP | Association | *OR* | 95% CI | *p* Value |
|---|---|---|---|---|---|
| Headaches | *CFH* rs1329428 | C/T vs. C/C | 0.24 | [0.089, 0.616] | .003 |
| | *CFH* rs1329428 | C/T + T/T vs. C/C | 0.25 | [0.099, 0.649] | .004 |
| Visual disturbances | *CFH* rs1329428 | C/T vs. C/C | 0.38 | [0.153, 0.918] | .03 |
| | *CFH* rs1329428 | C/T + T/T vs. C/C | 0.43 | [0.185, 0.987] | .05 |
| | *S100A8* rs3795391 | C/C + C/T vs. T/T | 0.07 | [0.009, 0.541] | .01 |
| | *F11R* rs7546890 | C/T + T/T vs. C/C | 0.36 | [0.144, 0.887] | .03 |
| Fatigue/tiredness | *S100A8* rs3795391 | C/C + TT vs.T/T | 0.28 | [0.096, 0.821] | .02 |
| Nausea and/or vomiting | *S100A8* rs3795391 | C/C + TT vs.T/T | 0.34 | [0.122, 0.953] | .04 |
| | *FSTL3* rs1046253 | G/A vs. A/A | 12.8 | [1.249, 130.191] | .03 |
| Indigestion/heartburn | *F11R* rs11265544 | C/C vs. A/A | 5.4 | [1.065, 27.754] | .04 |
| Palpitations | *F11R* rs836 | A/G vs. A/A | 0.04 | [0.004, 0.509] | .01 |
| Shortness of breath | *F11R* rs836 | A/A + A/G vs. G/G | 2.3 | [1.014, 5.170] | .05 |
| | *FSTL3* rs28364866 | T/C + T/T vs. C/C | 2.5 | [1.033, 6.218] | .04 |
| Back pain | *CFH* rs10922096 | C/T + T/T vs. C/C | 0.37 | [0.154, 0.887] | .03 |
| Chest pain | *CFH* rs10922096 | C/T + T/T vs. C/C | 0.28 | [0.091, 0.884] | .03 |

*Note.* CI = confidence interval; *OR* = odds ratio; SNP = single-nucleotide polymorphism.

was absent. A few symptoms were associated with increased likelihood by genotype. Nausea and/or vomiting was nearly 13 times as likely in women with G/A genotype in rs1046253. Indigestion/heartburn was over 5 times more likely with the C/C genotype in rs11265544. Shortness of breath was more than twice as likely with the absence of G/G in rs836 and was also 2.5 more likely in the absence of C/C genotype in rs28364866.

## Discussion

In this pilot study, we aimed to explore women's symptoms by gestational age in preeclampsia pregnancies and to examine associations of these symptoms with SNPs in novel candidate genes identified early in pregnancies of women who subsequently developed preeclampsia (Founds et al., 2009). This is the first study to look at symptoms across gestation in preeclampsia rather than focusing only on the late symptoms prodromal to potentially lethal end organ deterioration. The findings add quantitative support for the results of previous nursing research of women's symptoms in preeclampsia (Atterbury et al., 1998; Black, 2007; Kidner & Flanders-Stepans, 2004). At the same time, this is the first study to demonstrate the feasibility of associating symptoms in preeclampsia with SNPs in preeclampsia candidate genes. These data indicate that there are interesting symptom–genotype associations in preeclampsia that warrant further examination.

The TPR participants facilitated the success of this pilot collaboration through the Preeclampsia Foundation's online community, in which members are drawn together by surviving the adverse effects of the hypertensive disorders in pregnancy. These women were willing to enroll as active participants in TPR research. The early average gestational age of participants' births in the present study correlated with prior findings regarding early and severe types of hypertensive disorders of pregnancy (ACOG, 2013), which may have motivated research participation. Medical records submissions may have been constrained by the time frame of the project's funding; however, the records available for review revealed 100% concordance of documented diagnosis with self-report of a hypertensive disorder, with about half of the participants reporting the same type or severity of preeclampsia as was found in the medical records. Since we conducted this initial study, enrollment in TPR has grown 109%, to 3,470 participants, many with additional medical record validation completed.

The seriousness of the consequences of severe preeclampsia and HELLP syndrome necessitates that clinicians be able to differentiate symptoms that are common in pregnancy from symptoms of high-risk hypertensive disorders of pregnancy. All but one of our participants reported symptom items in the survey (Table 2). We identified the classical late prodromal symptoms of headache, visual disturbances, and epigastric/right upper quadrant pain in worsening preeclampsia in this group of women with frequencies similar to or higher than in prior descriptive research. More than half (51%) reported headaches in the third trimester, while 47.6% reported visual disturbances, 31.9% reported indigestion/heartburn, and 71.4% reported abdominal pain in the same trimester. In previous studies, 33–61% of women with preeclampsia reported headaches (Audibert, Friedman, Frangieh, & Sibai, 1996; Martin et al., 1999; National High Blood Pressure Education Program Working Group [NHBPEP], 1990; Sibai et al., 1993), 17% reported visual disturbances (Audibert et al., 1996), and 40–90% of women with hypertensive complications reported right upper quadrant or epigastric pain (Audibert et al., 1996; Martin et al., 1999; NHBPEP, 1990; Rath, Loos, Kuhn, & Graeff, 1990; Sibai et al., 1993; Weinstein, 1985).

The expanded survey we used for this study was more extensive than a checklist that had been previously validated for 11 symptoms of worsening preeclampsia or gestational hypertension (Black, 2007; Black & Morin, 2014). Women in the current study tended to have more severe disease, and there were no control or mild case groups. The frequency of

warning symptoms in the third trimester was similar to or higher than Black's results. In those two studies (Black, 2007; Black & Morin, 2014), persistent headaches occurred in 45.8% of women with worsening/severe hypertensive disorder, which was not significantly different than the 31.7% the researchers reported with this symptom in mild disease ($p = .16$). The 42.4% of women with severe disease who reported epigastric pain was not significantly different than the 34.1% of women who reported it with mild hypertensive disorder. Authors also found no significant differences between women with severe versus mild disease for blurred vision (33.9% vs. 19.5%, respectively; $p = .12$) or scotomata (32.2% vs. 9.5%, respectively; $p = .16$). In the present study, however, 32% of participants reported vertigo and 43.3% reported inability to concentrate in third trimester preeclampsia, which differs somewhat from the 47.5% and 39.0%, respectively, with worsening/severe preeclampsia who reported these symptoms in other studies (Black, 2007; Black & Morin, 2014). In the present study, we also provide quantitative data on the symptoms of back pain, excessive fatigue, shortness of breath, and not feeling right, which prior qualitative research had reported (Kidner & Flanders-Stepans, 2004).

In a recent nursing study, researchers used the Memorial Symptom Assessment Scale (MSAS) to evaluate the general symptom experience of women who were at 36 weeks or more of gestation and were low risk of pregnancy complications (Beebe, Gay, Richoux, & Lee, 2017). The MSAS, which consists of 32 items, had not previously been utilized with pregnant women. The Cronbach's α was .88 for the total score in a sample of 151 participants (Beebe et al., 2017). Sleep difficulties was the most common symptom reported, whereas in the current study of women with preeclampsia, sleep difficulties was the seventh most frequently reported, while swelling was reported as the most frequent.

One unique contribution of this pilot project is the advancement of symptom science (Cashion & Grady, 2015) in pregnancy. The results initiate the assessment of symptom patterns experienced across gestation in preeclampsia, demonstrating that holistic phenotyping of an adverse pregnancy outcome is feasible. Symptom phenotyping supports the identification of distressing antenatal symptoms that can influence the quality of life during pregnancy, maternal–fetal health, child growth and development, and family function (Betts, Williams, Najman, & Alati, 2014; Field, 2017a, 2017b).

Another critical and novel aspect of this pilot study is the demonstration of the feasibility of associating subjective symptom phenotype with genotype in preeclampsia. About half of the symptoms studied were associated with SNPs in novel preeclampsia CVS candidate genes (Founds et al., 2009). These findings are the first known associations of SNPs with warning symptoms in preeclampsia. Visual disturbances were associated with three SNPs: rs1329428, rs3795391, and rs7546890. Headaches were also associated with rs1329428. Indigestion/heartburn was associated with rs11265544, but it is unclear whether this symptom is related to epigastric pain per se. The potential for groups of SNPs to affect symptoms was demonstrated in visual disturbances, as mentioned, and nausea and/or vomiting, which was associated with rs3795391and rs1046253. There was no association between headaches and SNPs in *FSTL3*. This finding was unexpected because of the previous linkage studies of migraines with this chromosomal region (Ophoff et al., 1996; Sanchez et al., 2008).

Most of the associations with SNPs indicated reduced likelihood of symptoms, perhaps implicating genotypes with protective effects for symptom experiences. The three SNPs associated with visual disturbances all decreased the likelihood of reporting this symptom. On the other hand, four SNPs—rs1046253, rs11265544, rs836, and rs28364866—were associated with increased likelihood of symptoms in preeclampsia. Shortness of breath was more than twice as likely in the presence of each of the two SNPs, rs836 and rs28364866. These data may indicate the occurrence of symptoms as complex genetic traits (Founds et al., 2008; 2012).

### Limitations

Limitations of this pilot project included sample size and the potential for selection bias. The lack of adjustments for multiple comparisons may have been offset by a stringent *p* value for the selection of associations to submit to regression modeling. The small sample led to wide CIs for the *OR*s. The self-selected sample was not representative of the general population. Nonetheless, the TPR participants allowed us to demonstrate feasibility and indicate a promising direction for follow up of these initial results.

### Future Directions

Characterizing symptom phenotype across gestation and determining associations with genotype is a promising new direction for future research. Adequately powered studies that include medical records data in both uncomplicated pregnancy and adverse outcomes such as preeclampsia will expand on the preliminary findings of this pilot project. Oncology nursing research may provide models for studying symptom clusters in association with genotypes (Doong et al., 2015; Fu et al., 2016). Monitoring changes in symptom phenotype may eventually promote prevention and early detection of pregnancy complications, signaling the need for targeted therapeutic and clinical interventions (Cashion & Grady, 2015). The MSAS may be a useful instrument for broadening pregnancy symptom phenotypes (Beebe et al., 2017).

## Conclusions

In this successful collaboration with the Preeclampsia Foundation and TPR, we found that symptoms may feasibly be associated with candidate genes discovered in the first trimester placental tissues of preeclampsia pathogenesis (Founds et al., 2009). Follow up to validate these intriguing findings could pave the way for more holistic phenotyping of women during pregnancy to inform subgroups susceptible to particular

pathologies in preeclampsia. Identification of earlier symptoms or symptom patterns could signal the need for increased frequency and quality of surveillance with earlier intervention for mother and fetus. This approach could optimize health in pregnancy and later life for mothers and offspring through prediction, prevention, and early precision nursing care.

## Authors' Note

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## Authors' Contribution

Sandra A. Founds contributed to conception, design, acquisition, analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy. Eleni Tsigas contributed to conception, acquisition, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy. Dianxu Ren contributed to analysis and interpretation; drafted the manuscript; and gave final approval. M. Michael Barmada contributed to analysis and interpretation; drafted the manuscript; and gave final approval.

## Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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