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Polymorphisms in thrombophilia and renin–angiotensin system pathways, preterm delivery, and evidence of placental hemorrhage

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Abstract

Objective—To analyze functional polymorphisms in candidate genes (methylenetetrahydrofolate reductase (MTHFR)677C>T, MTHFR1298A>C, Factor 5 1691G>A (FVL), and angiotensinogen (AGT)-6G>A) in relation to a hypothesized placental hemorrhage pathway to preterm delivery (PTD).

Study Design—We assessed maternal genotypes, pregnancy outcomes, and placental pathology among 560 white and 399 black women recruited at midtrimester into a prospective cohort study (1998–2004). Odds of dominant genotypes were calculated for PTDs with (n=56) or without (n=177) evidence of placental hemorrhage (referent=term) using race-stratified polytomous logistic regression models.

Results—Among whites, FVL GA/AA and AGT(-6) GA/AA were both associated with hemorrhage-related PTDs (OR=4.8 [1.6, 14.2] and OR=3.8 [1.3, 10.5], respectively) but not other PTDs (ORs=1.2 and 0.9, respectively). FVL GA/AA was associated with PA (OR=5.8 [1.1, 30]) among white women. All results were null for MTHFR genotypes.

Conclusion—FVL and AGT variant genotypes were specifically associated with hemorrhage-related PTDs.

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Keywords

Preterm delivery; placental abruption; gene polymorphisms; placental pathology; thrombophilia

Introduction

Preterm delivery (PTD), defined as delivery prior to 37 completed weeks' gestation, complicates more than 10% of pregnancies in the United States, and contributes to the burden of neonatal morbidity and mortality.¹ Several pathways have been implicated in the etiology of PTD, including infection, stress, uterine distension, and uterine bleeding.¹ Placental abruption (PA), a rare pregnancy complication characterized by premature placental detachment, is strongly associated with PTD.² Moreover, other less severe concealed or subclinical placental hemorrhage evident only in placental pathology examinations may mark excess PTD risk.³ Although it has not been established whether all manifestations of placental hemorrhage belong to a common disease pathway, we hypothesize that preterm PA cases are the “tip of an iceberg,” the most extreme manifestations of a broader hemorrhage-related pathway.

Recurrence risk for PA is very high,^{4, 5} potentially suggesting a role for genetic factors. Hemostatic and hemodynamic dysregulation are two vascular mechanisms that have been implicated in PA risk. Two polymorphisms in the methylenetetrahydrofolate reductase (*MTHFR*) gene and one polymorphism in the Factor V (*F5*) gene (i.e. the Leiden variant, FVL) have been linked to a thrombophilic disposition. The angiotensinogen (*AGT*) gene has been studied with respect to hemodynamic dysregulation. Functional polymorphisms in these three genes have all previously been investigated in relation to PA (see review and meta-analysis⁶). Evidence of an association between FVL and PA has been fairly consistent, while evidence of associations between *MTHFR* or *AGT* variants and PA has been less convincing. Few studies have reported on these variants in relation to PTD, and results to date have not demonstrated significant associations⁷⁻¹². Other studies have attempted to link thrombophilias to vascular placental pathology findings among placentas from complicated deliveries,¹³⁻¹⁵ but these have not compared findings with term or uncomplicated deliveries. Given that some vascular function genotypes have been linked to PA, and PA is certainly linked to PTD, yet vascular function genotypes have not been convincingly linked to PTD or placental histopathology findings, we hypothesized that relevant genotypes might be associated with PTD subtypes defined by PA or hemorrhage-related placental pathology findings. We aimed to assess the associations between maternal gene polymorphisms and PTDs with clinical or pathology-based evidence of placental hemorrhage.

Materials and Methods

Study Protocol

The Pregnancy Outcomes and Community Health (POUCH) Study enrolled women with singleton pregnancies (15-27 weeks' gestation) from 52 clinics in five Michigan communities. The POUCH Study protocol was approved by the Institutional Review Boards of Michigan State University and Michigan Department of Community Health, and all women provided informed consent. Women were eligible for the POUCH Study if they had maternal serum alpha-fetoprotein (MSAFP) screening at 15-22 weeks, no pre-existing diabetes outside of pregnancy, age ≥ 15 years, English language proficiency, and no known congenital anomalies at the time of enrollment. Women with unexplained high MSAFP (≥ 2 multiples of the median) were oversampled, constituting 7% of the cohort (estimated 3.5% in general obstetric population). At enrollment, women provided a blood sample, participated in an in-person interview with a trained research nurse, and filled out a

questionnaire. From the interview and questionnaire, information was gathered on demographics, anthropometrics, substance use, prenatal vitamin use, and reproductive history. Women were asked to select their primary race or ethnic heritage from a list, e.g. “White or Caucasian” or “Black or African-American” (referred to as “white” and “black”).

Subcohort sample

A subcohort was selected for detailed study of biologic samples and medical records. The subcohort sample included all PTDs, all women with unexplained high MSAFP, and a stratified sample of term pregnancies with normal MSAFP; black women with term deliveries and normal MSAFP were oversampled. For the subcohort, medical records were abstracted in detail by study nurses with labor and delivery experience, placentas were examined by the study pathologist (PK Senagore), and genetic assays were conducted. The current analysis includes 996 white or black subcohort women with complete genotype and placental pathology data, and no placenta previa.

Pregnancy outcome measures

Gestational age was calculated based on last menstrual period; this estimate was replaced with an estimate from an ultrasound scan performed at <25 weeks' gestation if the two estimates differed by >14 days. PA was determined by consensus of three clinician-reviewers based on information in patient charts. The case definition required either (1) documented signs and symptoms consistent with PA (e.g. significant bleeding not attributable to dilatation, uterine pain or tenderness, fetal distress); or (2) retroplacental hematoma visualized on a prenatal ultrasound scan.

Placenta protocol

The pathologist was blinded to all clinical data including gestational age. For the gross examination, parallel slices were made through the placental disc 1 cm apart. The pathologist noted blood clots on the retromembranous, retroplacental, and disc cut surfaces, and adjacent tissue changes, i.e. dissecting hemorrhage, infarcted or compressed tissue, and red/brown tissue discoloration. *Disc-impacting blood clot* was defined as gross examination evidence of a retro- or intraplacental clot impacting adjacent tissue.

For the microscopic examination, nine placental tissue samples were examined: two membrane rolls, two sections of umbilical cord, and five full-thickness sections from the disc, 16 without pathologist knowledge of gross examination findings. Severe histologic chorioamnionitis was determined as previously described¹⁶. Microscopic vascular-related findings that fell within five constructs adapted from a diagnostic coding tool were recorded (Kelly R. et. al., American Journal of Epidemiology, in press). For our analysis, the construct of interest was “Maternal Vascular – Disturbance of Integrity” (MV-I), which included microscopic evidence of retroplacental blood with adjacent disc disruption/compression, decidual hemorrhage in the basal plate, and decidual hemosiderin-like pigment in the membranes or basal plate. High MV-I scores (i.e. the top quintile based on the distribution among term, normal MSAFP deliveries) were previously shown to correlate with PTD risk. The MV-I construct was selected to serve not as a ‘diagnostic instrument,’ but rather, as a distributionally-defined measure that identifies a continuum of findings consistent with maternal vessel bleeding. In this study, we considered two thresholds of MV-I scores to serve as possible indicators atypical maternal vessel hemorrhage: the top decile and the top quintile.

Thus, *pathology-based evidence of placental hemorrhage* includes gross examination findings of a disc-impacting blood clot and/or high scores in the microscopic examination

MV-I construct. When PA cases are excluded, these findings are referred to as *subclinical evidence of placental hemorrhage*.

Genetic assays

DNA was prepared from peripheral blood using a Puregene (Gentra) kit. Four polymorphisms were assayed by Polymerase Chain Reaction (PCR) followed by restriction digestion with appropriate restriction enzymes. The G-6A promoter variation in *AGT* and the FVL variation in *F5* were detected using published protocols.^{17, 18} The C677T polymorphism in *MTHFR* employed primers C677T (sense) 5'-TGA AGG AGA AGG TGT CTG CGG GA-3', (antisense) 5'-GAC GAT GGG GCA AGT GAT TC-3' for PCR amplification followed by digestion with *HinfI* to produce 100 base pair (bp) and 19 bp fragments for the C allele and 78, 22 and 19 bp fragments for the T allele. The *MTHFR* A1298C variant assay employed primers A1298C (sense) 5'-TCT ACC TGA AGA GCA AGT CC-3', (antisense) 5'-CAC TTC CAG CAT CAC TCA CT-3', followed by *MboII* digestion of the PCR product to yield 72, 30, 28 and 20 bp products for the A allele and 100, 30, and 20 bp products for the C allele. Minor allele frequencies and deviation from Hardy-Weinberg equilibrium were calculated by race.

Analytic strategy

Analyses incorporate weights (inverse of sampling probability) using the SURVEY procedures in SAS (Statistical Analysis Software, Cary, NC) to account for the complex sampling scheme.

As a first step, we compared distributions of maternal characteristics to evidence of placental hemorrhage using a 3-level hierarchical variable: (1) PA, (2) subclinical evidence of placental hemorrhage (i.e. disc-impacting blood clots or the top decile of MV-I scores) or (3) none. Second, with PTD as the outcome, we estimated race-specific ORs for each variant assuming dominant models, i.e. women having 1 or 2 copies of the minor allele versus with women having 0 copies, except when zero cells prohibited analysis. This was repeated with PA as the outcome.

Next, we were interested in whether maternal genotypes were specifically associated with the hypothesized placental hemorrhage-related PTD pathway. To this end, race-specific polytomous logistic regression models were developed to compare two broad PTD subtypes with term deliveries: (1) PTDs with any evidence of placental hemorrhage (i.e. PA or subclinical evidence of placental hemorrhage), and (2) PTDs without any evidence of placental hemorrhage. This analysis was repeated after changing the threshold for defining a high MV-I score from the top decile to the top quintile. Although few cases of preterm PA were available, we performed another polytomous regression analysis after separating PTDs with PA from PTDs with subclinical evidence of placental hemorrhage. *MTHFR* analyses were repeated after excluding women who were taking prenatal vitamins preconceptionally. All analyses were repeated after excluding women with severe histologic chorioamnionitis.

Based on a type I error rate of 0.05, the POUCH Study was originally designed to have at least 65% power to detect ORs of 2.0 in the subcohort for exposures present in 20% of the population, when considering PTD subtypes that occurred in at least 5-7% of the population. The current analysis focuses on a narrower PTD subtype, which occurred in only 2% of the population. Variation in allele frequencies and the necessity to stratify on race resulted in 80% power to detect ORs ranging from 3.0 to 6.3 depending on genotype and race (with the exception of FVL and *AGT* in blacks, where extreme genotype distributions precluded most analyses.).

Results

Race-specific minor allele frequencies are shown in Table 1. The minor alleles for *MTHFR* C677T, *MTHFR* A1298C, and FVL were more common in whites than blacks. For *AGT*, the G allele predominated in whites while the A allele predominated in blacks. Hardy-Weinberg equilibrium was not violated for any allele in either race.

The distributions of maternal characteristics in the study sample and the prevalence of PA, subclinical evidence of placental hemorrhage, and no evidence of placental hemorrhage by these characteristics are detailed in Table 2. The weighted incidence of PTD was 10.7%. PA was clinically diagnosed in 30 women (2.1%), and subclinical evidence of placental hemorrhage was present in 9.5% (as defined based in part on a distributional cutpoint), and these were both overrepresented among PTDs (7.3% and 12.3%, $P<.05$). Other maternal characteristics did not differ significantly across the outcome categories. Potential confounders of the genotype-pregnancy outcome relations would need to be associated with both the genotypes and the outcomes. None of the maternal characteristics met these criteria, thus these were not used to adjust subsequent analyses.

Table 3 shows dominant genotype models for PTD. There were no significant associations between any genotype and PTD. There was an association between FVL GA/AA and PA (OR=5.8, 95% CI 1.1, 30.3) among white women (not in table).

Results of the polytomous logistic regression analysis of PTD subtypes defined broadly as those with or without any evidence of placental hemorrhage (i.e. PA and/or pathology-based evidence of placental hemorrhage) are presented in Table 4. Among white women, those with PTD and evidence of placental hemorrhage were more likely than women with term deliveries to have the FVL GA/AA genotypes (OR=4.8, 95% CI 1.6, 14.2) or the *AGT*(-6) GA/AA genotypes (OR=3.8, 95% CI 1.3, 10.5); the corresponding ORs for PTDs without evidence of placental hemorrhage were close null (1.2 and 0.9, respectively).

After changing the threshold of the MV-I construct from the top decile to the top quintile (which identified 1/3 of all PTDs as having evidence of hemorrhage), results were attenuated but still significant for FVL (OR=3.2, 95% CI 1.3, 8.3) but weaker for *AGT* (OR=1.6, 95% CI 0.8, 3.1). Results for both *MTHFR* variant genotypes were null in both blacks and whites. Repeating the *MTHFR* analyses in women who were not taking prenatal vitamins prior to conception produced similar null results.

To ensure that infection-related PTDs were not obscuring true associations between genotypes and hemorrhage-related PTDs, the models were also run after excluding women with severe histologic chorioamnionitis from the dataset, and results were similar (not shown).

Finally, we subdivided PTDs with evidence of placental hemorrhage into those with PA and those with subclinical evidence of placental hemorrhage (Table 5). FVL GA/AA was associated with both (PTD with PA OR=5.4, 95% CI 1.0, 28.2; PTD with subclinical evidence of hemorrhage OR=4.4, 95% CI 1.1, 16.9). The *AGT* GA/AA genotype was not significantly associated with PTD with PA (OR=2.5, 95% CI 0.5, 13.1), but was associated with PTDs with subclinical evidence of hemorrhage (OR=5.1, 95% CI 1.5, 17.2).

Comment

In this study, we investigated *MTHFR* C677T, *MTHFR* A1298C, FVL, and *AGT* G-6A variant genotypes in relation to PTD subtypes defined by evidence of placental hemorrhage. Although none of the genotypes were associated with PTD overall, when we brought

information on delivery timing and placental hemorrhage together, we found that FVL GA/AA and *AGT*(-6) GA/AA genotypes were both associated with PTDs having evidence of placental hemorrhage in white women. This association was not attributable solely to clinically-detected PA cases. Notably, the observed effects were specific in that the ORs for PTDs without any evidence of placental hemorrhage were very close to the null value.

A recent meta-analysis found a significant association between FVL and PA, based on 10 studies.⁶ Our results corroborate a strong association between FVL and placental abruption risk, and contribute the additional finding that FVL is associated with a specific subset of PTDs that have more broadly-defined evidence of placental hemorrhage, including subclinical hemorrhage identified through placental pathology exams. Normal pregnancy alters hemostasis, shifting the balance to a relatively hypercoagulable state.¹⁹ Inherited thrombophilias may exacerbate this shift and predispose women to develop clots at the maternal-fetal interface. Although exact mechanisms are unknown, these clots may somehow facilitate rupture of decidual blood vessels, resulting in decidual hemorrhage and possibly also premature placental detachment. However, subclinical decidual hemorrhage might lead to PTD via other mechanisms.^{20, 21}

We found that *AGT*(-6) GA/AA was associated with PTD with evidence of placental hemorrhage among white women. The *AGT*(-6) promoter polymorphism is in strong linkage disequilibrium with the *AGT* M235T polymorphism.²² One study conducted in Mexico identified an association between *AGT* M235T and preterm premature rupture of membranes¹², and only two studies have reported on *AGT* polymorphisms and abruption risk, with conflicting results.^{23, 24} This variant has also been associated with preeclampsia²⁵ and hypertension outside of pregnancy.²⁶ PA has been consistently linked to high blood pressure,²⁷⁻³⁰ yet it is unknown whether the association between *AGT* genotypes and PA observed in one large study²⁴ was attributable to hypertension. We speculate that local effects of the renin-angiotensin system in the decidual spiral arteries³¹ may have implications for hemorrhage-related PTD risk, possibly in the absence of systemic hypertension.

At the POUCH study's outset, *MTHFR* was a promising candidate gene for vascular diseases, and its variants have received a great deal of research attention in the obstetrics literature since that time. We identified no associations between *MTHFR* genotypes and placental abruption or hemorrhage-related PTD in black or white women. These null results add to accumulating evidence that these variants may not be strongly related to pregnancy outcomes,^{6-7, 9, 32-34, 36-37} although a few studies have identified positive associations with PA.^{10-11, 35} While there is strong biologic rationale for a role of *MTHFR* variants in poor pregnancy outcomes through a thrombophilia pathway, variant genotypes may only result in hyperhomocysteinemia with a thrombotic tendency among individuals with low folate intake. POUCH Study enrollment began after mandatory grain fortification in the United States, and most women reported taking prenatal vitamins, thus folate deficiency was probably rare. Direct measures of folate and homocysteine status were not available. Results did not differ meaningfully after we excluded women who took prenatal vitamins prior to conception, but we cannot rule out a stronger effect in populations with substantially lower folate intake.

The results of this study suggest additional avenues for research. Variants in other genes implicated in thrombophilias, e.g. Factor 2 (prothrombin) and plasminogen activator inhibitor-1, and other variants in renin-angiotensin system genes, might be associated with PTD with placental hemorrhage. Furthermore, given that the placenta has a fetal genotype, consideration of fetal DNA and maternal-fetal genotype interactions may shed additional light on risk of both PA and PTD.

The most important strength of this study is the objective assessment of gross and microscopic placental pathology in a large sample of preterm and term deliveries, which enabled us to investigate genotypes in relation to etiologically relevant PTD subgroups. At least three studies have attempted to link maternal or fetal thrombophilias to specific placental lesions within pregnancies complicated by PA, preeclampsia, or fetal growth restriction; however, none of these studies included a comparison group of placentas from uncomplicated pregnancies.¹³⁻¹⁵ Given the prospective design, the POUCH Study is limited by a relatively small number of PTDs with PA or other evidence of placental hemorrhage. Some identified statistically significant relations, such as that between FVL or AGT genotypes and hemorrhage-related PTDs in white women, may be accompanied by inflated ORs, as is often the case for newly discovered associations³⁸. While we believe the results of this study are valid and biologically plausible, caution is warranted in interpreting the magnitude of the observed associations in light of the acknowledged power limitations. No adjustments were made for multiple comparisons and it is likely that some identified associations would lose statistical significance if we did so; however, in our view this practice is unwarranted in the context of *a priori* selection of a modest number of candidate genes because it inappropriately increases the probability of type II error³⁹.

Polymorphisms related to hemostasis and hemodynamics may be associated with PTD through pathways involving disrupted vascular integrity, ie, PA or subclinical pathology-based evidence of placental hemorrhage. Pending replication of these findings in other studies, it may be possible to identify a set of upstream markers including these maternal gene polymorphisms that discriminates women at highest risk of vascular-mediated PTD or overt PA, and to implement preventive measures to improve outcomes for both the mother and child.

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References

1. Green NS, Damus K, Simpson JL, et al. Research agenda for preterm birth: recommendations from the March of Dimes. *Am J Obstet Gynecol* 2005;193:626–35. [PubMed: 16150253]
2. Ananth CV, Berkowitz GS, Savitz DA, Lapinski RH. Placental abruption and adverse perinatal outcomes. *Jama* 1999;282:1646–51. [PubMed: 10553791]
3. Salafia CM, Lopez-Zeno JA, Sherer DM, Whittington SS, Minior VK, Vintzileos AM. Histologic evidence of old intrauterine bleeding is more frequent in prematurity. *Am J Obstet Gynecol* 1995;173:1065–70. [PubMed: 7485294]
4. Ananth CV, Peltier MR, Chavez MR, Kirby RS, Getahun D, Vintzileos AM. Recurrence of Ischemic Placental Disease. *Obstet Gynecol* 2007;110:128–133. [PubMed: 17601907]
5. Misra DP, Ananth CV. Risk factor profiles of placental abruption in first and second pregnancies: heterogeneous etiologies. *J Clin Epidemiol* 1999;52:453–61. [PubMed: 10360341]
6. Zdoukopoulos N, Zintzaras E. Genetic risk factors for placental abruption: a HuGE review and meta-analysis. *Epidemiology* 2008;19:309–23. [PubMed: 18277167]

7. Engel SM, Olshan AF, Siega-Riz AM, Savitz DA, Chanock SJ. Polymorphisms in folate metabolizing genes and risk for spontaneous preterm and small-for-gestational age birth. *Am J Obstet Gynecol* 2006;195:1231 e1–11. [PubMed: 17074544]
8. Kocher O, Cirovic C, Malynn E, et al. Obstetric complications in patients with hereditary thrombophilia identified using the LCx microparticle enzyme immunoassay: a controlled study of 5,000 patients. *Am J Clin Pathol* 2007;127:68–75. [PubMed: 17145624]
9. Resch B, Gallistl S, Kutschera J, Mannhalter C, Muntean W, Mueller WD. Thrombophilic polymorphisms--factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T mutations--and preterm birth. *Wien Klin Wochenschr* 2004;116:622–6. [PubMed: 15515881]
10. Stonek F, Hafner E, Philipp K, Hebler LA, Bentz EK, Tempfer CB. Methylenetetrahydrofolate reductase C677T polymorphism and pregnancy complications. *Obstet Gynecol* 2007;110:363–8. [PubMed: 17666612]
11. Valdez LL, Quintero A, Garcia E, et al. Thrombophilic polymorphisms in preterm delivery. *Blood Cells Mol Dis* 2004;33:51–6. [PubMed: 15223011]
12. Valdez-Velazquez LL, Quintero-Ramos A, Perez SA, et al. Genetic polymorphisms of the renin-angiotensin system in preterm delivery and premature rupture of membranes. *J Renin Angiotensin Aldosterone Syst* 2007;8:160–8. [PubMed: 18205094]
13. Ariel I, Anteby E, Hamani Y, Redline RW. Placental pathology in fetal thrombophilia. *Hum Pathol* 2004;35:729–33. [PubMed: 15188139]
14. Many A, Schreiber L, Rosner S, Lessing JB, Eldor A, Kupferminc MJ. Pathologic features of the placenta in women with severe pregnancy complications and thrombophilia. *Obstet Gynecol* 2001;98:1041–4. [PubMed: 11755551]
15. Mousa HA, Alfirevic Z. Do placental lesions reflect thrombophilia state in women with adverse pregnancy outcome? *Hum Reprod* 2000;15:1830–3. [PubMed: 10920112]
16. Holzman C, Lin X, Senagore P, Chung H. Histologic chorioamnionitis and preterm delivery. *Am J Epidemiol* 2007;166:786–94. [PubMed: 17625222]
17. Molina-Molina M, Xaubet A, Li X, et al. Angiotensinogen gene G-6A polymorphism influences idiopathic pulmonary fibrosis disease progression. *Eur Respir J* 2008;32:1004–8. [PubMed: 18508830]
18. Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Eisenberg PR, Miletich JP. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. *N Engl J Med* 1995;332:912–7. [PubMed: 7877648]
19. Brenner B. Haemostatic changes in pregnancy. *Thromb Res* 2004;114:409–14. [PubMed: 15507271]
20. Krikun G, Huang ST, Schatz F, Salafia C, Stocco C, Lockwood CJ. Thrombin activation of endometrial endothelial cells: a possible role in intrauterine growth restriction. *Thromb Haemost* 2007;97:245–53. [PubMed: 17264954]
21. Lockwood CJ, Toti P, Arcuri F, et al. Mechanisms of abruption-induced premature rupture of the fetal membranes: thrombin-enhanced interleukin-8 expression in term decidua. *Am J Pathol* 2005;167:1443–9. [PubMed: 16251427]
22. Jeunemaitre X, Inoue I, Williams C, et al. Haplotypes of angiotensinogen in essential hypertension. *Am J Hum Genet* 1997;60:1448–60. [PubMed: 9199566]
23. Hillermann R, Carelse K, Gebhardt GS. The Glu298Asp variant of the endothelial nitric oxide synthase gene is associated with an increased risk for abruptio placentae in preeclampsia. *J Hum Genet* 2005;50:415–9. [PubMed: 16059745]
24. Zhang XQ, Craven C, Nelson L, Varner MW, Ward KJ. Placental abruption is more frequent in women with the angiotensinogen Thr235 mutation. *Placenta* 2007;28:616–9. [PubMed: 17116328]
25. Medica I, Kastrin A, Peterlin B. Genetic polymorphisms in vasoactive genes and preeclampsia: a meta-analysis. *Eur J Obstet Gynecol Reprod Biol* 2007;131:115–26. [PubMed: 17112651]
26. Sethi AA, Nordestgaard BG, Tybjaerg-Hansen A. Angiotensinogen gene polymorphism, plasma angiotensinogen, and risk of hypertension and ischemic heart disease: a meta-analysis. *Arterioscler Thromb Vasc Biol* 2003;23:1269–75. [PubMed: 12805070]

27. Ananth CV, Savitz DA, Williams MA. Placental abruption and its association with hypertension and prolonged rupture of membranes: a methodologic review and meta-analysis. *Obstet Gynecol* 1996;88:309–18. [PubMed: 8692522]
28. Hogberg V, Rasmussen S, Irgens LM. The effect of smoking and hypertensive disorders on abruptio placentae in Norway 1999–2002. *Acta Obstet Gynecol Scand* 2007;86:304–9. [PubMed: 17364304]
29. Zetterstrom K, Lindeberg SN, Haglund B, Hanson U. Maternal complications in women with chronic hypertension: a population-based cohort study. *Acta Obstet Gynecol Scand* 2005;84:419–24. [PubMed: 15842204]
30. Williams MA, Mittendorf R, Monson RR. Chronic hypertension, cigarette smoking, and abruptio placentae. *Epidemiology* 1991;2:450–3. [PubMed: 1790199]
31. Morgan T, Craven C, Nelson L, Lalouel JM, Ward K. Angiotensinogen T235 expression is elevated in decidual spiral arteries. *J Clin Invest* 1997;100:1406–15. [PubMed: 9294106]
32. Ananth CV, Peltier MR, De Marco C, et al. Associations between 2 polymorphisms in the methylenetetrahydrofolate reductase gene and placental abruption. *Am J Obstet Gynecol* 2007;197:385 e1–7. [PubMed: 17904970]
33. Jaaskelainen E, Keski-Nisula L, Toivonen S, et al. MTHFR C677T polymorphism is not associated with placental abruption or preeclampsia in Finnish women. *Hypertens Pregnancy* 2006;25:73–80. [PubMed: 16867914]
34. Karakantza M, Androutsopoulos G, Mougou A, Sakellariopoulos G, Kourounis G, Decavalas G. Inheritance and perinatal consequences of inherited thrombophilia in Greece. *Int J Gynaecol Obstet* 2008;100:124–9. [PubMed: 17963764]
35. Nurk E, Tell GS, Refsum H, Ueland PM, Vollset SE. Associations between maternal methylenetetrahydrofolate reductase polymorphisms and adverse outcomes of pregnancy: the Hordaland Homocysteine Study. *Am J Med* 2004;117:26–31. [PubMed: 15210385]
36. Parle-McDermott A, Mills JL, Kirke PN, et al. MTHFD1 R653Q polymorphism is a maternal genetic risk factor for severe abruptio placentae. *Am J Med Genet A* 2005;132:365–8. [PubMed: 15633187]
37. Prochazka M, Lubusky M, Slavik L, et al. Frequency of selected thrombophilias in women with placental abruption. *Aust N Z J Obstet Gynaecol* 2007;47:297–301. [PubMed: 17627684]
38. Ioannidis JP. Why most discovered true associations are inflated. *Epidemiology* 2008;19:640–8. [PubMed: 18633328]
39. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990;1:43–6. [PubMed: 2081237]

Table 1
Race-specific minor allele frequencies for measured gene polymorphisms among 560 white and 399 black women

Polymorphism	dbSNP ID	Minor allele frequency	
		White	Black
MTHFR(677) C>T	rs1801131	0.344	0.102
MTHFR(1298) A>C	rs1801131	0.317	0.19
FVL(1691) G>A	rs6025	0.028	0.004
AGT(-6) G>A	rs5051	0.422	0.834

Table 2
Maternal characteristics of subcohort sample, and prevalence of placental abruption, subclinical evidence of placental hemorrhage, and no evidence of placental hemorrhage

	Subcohort Sample		Evidence of Placental Hemorrhage					
			Placental Abruption		Subclinical Evidence*		None	
	N	% [†]	N	% [‡]	N	% [‡]	N	% [‡]
Preterm delivery <37 weeks	959	100.0	30	2.1	93	9.5	836	88.4
Yes	223	10.7	18	7.3	28	12.3	177	80.4
No	736	89.3	12	1.4	65	9.2	659	89.4
Race								
White	560	75.4	15	1.7	60	9.9	485	88.3
Black	399	24.6	15	3.1	33	8.2	351	88.7
Medicaid-insured								
Yes	516	45.7	18	2.1	45	7.7	453	90.2
No	442	54.3	12	2.0	48	11.1	382	86.9
Age in years								
<20	154	13.3	6	2.2	20	11.5	128	86.3
20-34	735	78.4	22	2.1	66	9.0	647	88.9
35+	70	8.3	2	1.4	7	11.3	61	87.3
Smoked during pregnancy								
Yes (includes quit prior to enrollment)	270	26.7	5	0.9	24	8.0	241	91.1
No	689	73.3	25	2.5	69	10.0	595	87.5
Cocaine use								
Ever	80	8.7	3	1.4	6	7.2	71	91.4
Never	879	91.3	27	2.1	87	9.7	765	88.2
Pre-pregnancy Body Mass Index (kg/m ²)								
<18.5	40	3.3	4	6.4	4	10.0	32	83.6
18.5 – 24.9	424	45.7	16	2.2	46	10.5	362	87.3
25-29.9	218	24.5	5	1.8	18	7.6	195	90.6

	Subcohort Sample	Evidence of Placental Hemorrhage							
		Placental Abruptio		Subclinical Evidence *		None			
		N	% [†]	N	% [‡]	N	% [‡]		
≥30		277	26.4	5	1.5	25	9.4	247	89.0
Pregnancy history									
Nulliparous		390	40.7	7	0.8	42	9.1	341	90.1
Parous, no prior Preterm delivery		437	49.5	15	2.8	42	10.2	380	87.0
Parous, prior Preterm delivery		131	9.8	8	3.7	9	7.5	114	88.8
Hypertension									
Yes (PE, PIH, or chronic)		97	8.7	4	4.2	15	14.3	78	81.5
None		862	91.3	26	1.9	78	9.0	758	89.1
Prenatal vitamin use									
Started prior to pregnancy		120	14.4	3	0.9	16	14.0	101	85.1
Started during first trimester		587	63.1	22	2.7	55	8.7	510	88.6
Started prior to study enrollment during second trimester		128	11.0	3	1.3	11	9.0	114	89.7
Not taking at enrollment (15-27 weeks)		124	11.6	2	0.9	11	8.8	111	90.3

* Subclinical evidence of placental hemorrhage defined as presence of a disc-impacting blood clot in the gross placental examination or a microscopic vascular – disturbance of integrity score in the top decile as observed in the microscopic placental exam.

[‡] Column percent, weighted using inverse of sampling probability to reflect distribution in POUCH cohort.

[‡] Row percent, weighted using inverse of sampling probability to reflect distribution in POUCH cohort.

Table 3

Association between vascular function genotypes (dominant models) and preterm delivery among 560 white women and 399 black women

Race	Genotype	Preterm Delivery		
		Yes	No	OR* 95% CI
White	Total	152	408	
	MTHFR (677)			
	CC	62	174	1.0
	TC or TT	90	234	1.0 0.7, 1.5
	MTHFR (1298)			
	AA	70	189	1.0
	AC or CC	82	219	1.0 0.7, 1.5
	FVL (169I)			
	GG	140	386	1.0
	GA or AA	12	22	1.7 0.8, 3.7
Black	AGT(-6)			
	GG	50	136	1.0
	GA or AA	102	272	1.0 0.7, 1.6
	Total	71	328	
	MTHFR (677)			
	CC	57	265	1.0
	TC or TT	14	63	1.1 0.5, 2.0
	MTHFR (1298)			
	AA	48	220	1.0
	AC or CC	23	108	1.0 0.5, 1.7
	FVL (169I)			
	GG	71	325	
	GA or AA	0	3	
	AGT(-6)			
	GG	2	8	1.0
	GA or AA	69	320	0.8 0.2, 3.8

* Odds ratios (OR) and 95% confidence intervals (CI) from weighted (inverse of sampling probability) logistic regression models.

Table 4
Association between vascular function genotypes (dominant models) and PTD subtypes defined by presence or absence of evidence of hemorrhage compared with term deliveries among 560 white women and 399 black women

Race	Genotype	Term	Preterm Delivery Subtypes					
			With Evidence of Placental Hemorrhage*			Without Evidence of Placental Hemorrhage*		
			n	OR [†]	95% CI	n	OR [†]	95% CI
White	Total		408			124		
	MTHFR (677)							
	CC		174	1.0		49	1.0	
	TC or TT		234	0.8	0.4, 1.7	75	1.1	0.7, 1.7
	MTHFR (1298)							
	AA		189	1.0		60	1.0	
	AC or CC		219	1.7	0.7, 4.0	64	0.9	0.6, 1.4
	FVL (1691)							
	GG		386	1.0		117	1.0	
	GA or AA		22	4.8	1.6, 14.2	7	1.2	0.5, 3.0
Black	AGT(-6)							
	GG		136	1.0		45	1.0	
	GA or AA		272	3.8	1.3, 10.5	79	0.9	0.6, 1.3
	Total		328			53		
	MTHFR (677)							
	CC		265	1.0		41	1.0	
	TC or TT		63	0.5	0.1, 2.2	12	1.3	0.6, 2.6
	MTHFR (1298)							
	AA		220	1.0		34	1.0	
	AC or CC		108	0.6	0.2, 1.9	19	1.1	0.6, 2.1
	FVL (1691)							
	GG		325			53		
	GA or AA		3			0		
	AGT(-6)							

		Preterm Delivery Subtypes						
Term		With Evidence of Placental Hemorrhage *			Without Evidence of Placental Hemorrhage *			
		n	n	OR [†]	95% CI	n	OR [†]	95% CI
	Race	Genotype						
		GG	8	0			2	
		GA or AA	320	18			51	

* Evidence of placental hemorrhage from clinical data or placental pathology exams. Preterm deliveries with evidence of placental hemorrhage defined as preterm deliveries accompanied by placental abruption, disc-impacting blood clot observed in the gross pathology examination, or a score in the top decile of the microscopic vascular - disturbance of integrity construct in the microscopic placental pathology examination. Preterm deliveries with evidence of placental hemorrhage defined as all other preterm deliveries.

† Odds ratios (OR) and 95% confidence intervals (CI) from weighted (inverse of sampling probability) polytomous logistic regression models (term deliveries = referent).

Table 5
Association between vascular function genotypes (dominant models) and preterm delivery subtypes defined by placental abruption, subclinical evidence of placental hemorrhage, and no evidence of placental hemorrhage compared with term deliveries among white or black women

		Preterm Deliveries							
Race	Genotype	Term		With Placental Abruption		With Subclinical Evidence of Placental Hemorrhage*		With No Evidence of Placental Hemorrhage	
		n	n	OR [†]	95% CI	n	OR [†]	n	OR [†]
White	Total	408	10			18		124	
	MTHFR (677)								
	CC	174	6			7		49	
	TC or TT	234	4	0.4	0.1, 1.3	11	1.2	75	1.1
									0.7, 1.7
	MTHFR (1298)								
	AA	189	4			6		60	
	AC or CC	219	6	1.4	0.4, 5.3	12	1.9	64	0.9
									0.6, 1.4
	FVL (1691)								
Black	GG	386	8			15		117	
	GA or AA	22	2	5.4	1.0, 28.2	3	4.4	7	1.2
									0.5, 3.0
	AGT(-6)								
	GG	136	2			3		45	
	GA or AA	272	8	2.5	0.5, 13.1	15	5.1	79	0.9
									0.6, 1.3
	Total	328	8			10		53	
	MTHFR (677)								
	CC	265	7			9		41	
	TC or TT	63	1	0.4	0.04, 3.4	1	0.5	12	1.3
									0.6, 2.6
	MTHFR (1298)								
	AA	220	7			7		34	
	AC or CC	108	1	0.2	0.02, 1.6	3	0.9	19	1.1
									0.6, 2.1
	FVL (1691)								
	GG	325	8			10		53	
	GA or AA	3	0			0		0	

		Preterm Deliveries								
Term		With Placental Abruptio		With Subclinical Evidence of Placental Hemorrhage*		With No Evidence of Placental Hemorrhage				
Race	Genotype	n	OR [†]	95% CI	n	OR [†]	95% CI	n	OR [†]	95% CI
AGT(-6)										
	GG	8	0		0			2		
	GA or AA	320	8		10			51		

* Subclinical evidence of placental hemorrhage was defined as presence of a disc-impacting blood clot in the gross placental examination or a score in the top decile of the microscopic vascular—disturbance of integrity construct in the microscopic placental examination, among women who did not have placental abruptio.

† Odds ratios (OR) and 95% confidence intervals (CI) from weighted (inverse of sampling probability) polytomous logistic regression models (term deliveries = referent).