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EVIDENCE OF PLACENTAL HEMORRHAGE: RELATIONS WITH PRETERM DELIVERY, POLYMORPHISMS IN VASCULAR FUNCTION GENES, AND INTRAUTERINE INFECTION

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EVIDENCE OF PLACENTAL HEMORRHAGE: RELATIONS WITH PRETERM DELIVERY, POLYMORPHISMS IN VASCULAR FUNCTION GENES, AND INTRAUTERINE INFECTION

By

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ABSTRACT

EVIDENCE OF PLACENTAL HEMORRHAGE: RELATIONS WITH PRETERM DELIVERY, POLYMORPHISMS IN VASCULAR FUNCTION GENES, AND INTRAUTERINE INFECTION

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Julia Marie Warner Gargano

Bleeding has been identified as a major etiologic pathway to preterm delivery (PTD). We hypothesized that placental abruption may be an extreme manifestation of this pathway, while early pregnancy vaginal bleeding and placental pathology findings may identify early or subclinical manifestations, respectively. We aimed (1) to evaluate multiple indicators of placental hemorrhage as potential components of a common bleeding pathway, (2) to assess the associations between maternal gene polymorphisms in thrombophilia and renin-angiotensin system pathways and PTD subtypes defined by evidence of placental hemorrhage, and (3) to evaluate risk of histologic chorioamnionitis and clinical chorioamnionitis in relation to early and late evidence of placental hemorrhage.

A subcohort (N=1371) of pregnant women were recruited at midtrimester (15-27 weeks' gestation) as part of a prospective cohort study (1998-2004). Data were ascertained by interviews conducted at enrollment, detailed medical chart abstraction, maternal blood assays and placental pathology examinations. We analyzed data on 996 black or white subcohort women who had complete placenta data and did not have placenta previa. Data on functional polymorphisms in candidate genes (methylene-tetrahydrofolate reductase (MTHFR) C677T, MTHFR A1298C, Factor V G1691A (Leiden, FVL), and angiotensinogen (AGT) G-6A) were available on 959 of these

women. Information derived from the gross and microscopic placental examinations included histologic chorioamnionitis, disc-impacting blood clots, and Maternal Vascular – Disturbance of Integrity (MV-I) scores.

Four manifestations of placental hemorrhage, i.e. placental abruption, disc-impacting blood clots, top quintile of MV-I scores, and first trimester bleeding, differed in their associations with some maternal characteristics and were not highly concordant with one another. Subclinical evidence of placental hemorrhage identified through placental pathology exams was associated with increased odds of PTD, particularly PTD at <35 weeks, after accounting for clinically evident bleeding in a multivariable model. Women who were heterozygous for FVL or the AGT -6 A allele were at increased risk of PTD with evidence of placental hemorrhage, whereas they were not at increased risk of PTD without evidence of placental hemorrhage. Placental abruption and disc-impacting blood clots were associated with clinical chorioamnionitis, while bleeding in the first and second trimesters was associated with the histologic chorioamnionitis, although there was evidence of an interaction with delivery timing for histologic chorioamnionitis.

Multiple clinical and subclinical indicators of placental hemorrhage are related to PTD. However, because associations with maternal characteristics, gene polymorphisms, and intrauterine infection differed among measures of placental hemorrhage we conclude that heterogeneity exists even within the "bleeding pathway." PTD may be marked by early or late bleeding for a number of reasons. Greater insight into bleeding-related pathways may be achieved by incorporating information on subclinical hemorrhage.

DEDICATION

To Amelia, with love

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It has been a great privilege to work on the POUCH Study. I cannot name all the people who contributed to this endeavor, but I am aware that I stand on the shoulders of many in presenting study results. Here, I mention a few people who have directly contributed to this work. Dr. Karen Friderici and Katherine Jernigan were responsible for the genetic assays employed in this study. Dr. Rachel Fisher provided valuable critical feedback as I developed ideas that formed the foundation of this dissertation. Dr. Judith Suess, Dr. Nazish Siddiqi, and Lynn Thelen reviewed the charts of suspected placental abruption cases. Dr. Bertha Bullen and Crista Valentine have made the POUCH Study run smoothly, and have provided direct assistance and moral support.

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KEY TO ABBREVIATIONS

PTD	Preterm Delivery
OR	Odds Ratio
CI	Confidence Interval
BMI	Body Mass Index
HCA	Histologic Chorioamnionitis
CCA	Clinical Chorioamnionitis
MV-I	Maternal Vascular – Disturbance of Integrity
MSAFP	Maternal Serum Alpha-fetoprotein
MTHFR	Methylene tetrahydrofolate reductase
AGT	Angiotensinogen
FVL	Factor V Leiden

CHAPTER 1.

BACKGROUND LITERATURE AND AIMS

1.1. Preterm delivery subtypes and etiologic pathways

Preterm delivery, defined as delivery of a fetus prior to 37 completed weeks' gestation, increases risks of neonatal morbidity and mortality(1). Approximately 10% of singleton deliveries in the United States are preterm, and rates of preterm delivery have been increasing(2). Although many risk factors for preterm delivery have been identified, including African-American race, low socioeconomic status, alterations in vaginal flora, stress, low and high body mass index, substance abuse and short interpregnancy interval, accurate prediction of preterm delivery is not possible(3). The strongest predictor of preterm delivery is a history of preterm delivery in a prior pregnancy(4, 5).

Preterm delivery is an occurrence with many causes and multiple clinical presentations(6-13). Therefore, researchers have subdivided preterm deliveries into relatively homogeneous subtypes to improve specificity of associations with candidate risk factors(5, 11, 14-17). Many studies, including those of the National Institutes of Child Health and Development Maternal-Fetal Medicine Units, have classified preterm deliveries according to gestational age at delivery(18-20). Other classification schemes have relied on clinical presentation. For example, Meis divided preterm deliveries into 'medically indicated' (i.e., those associated with placenta previa, placental abruption, antepartum hemorrhage, preeclampsia/eclampsia and renal disease regardless of whether they were induced), and spontaneous (i.e. all other preterm deliveries), and found that they differed with respect to their associations with maternal age, hemoglobin levels, and bacteriuria, but were similar with respect to many other risk factors.(15) More recent

studies have restricted the 'medically indicated' category to those deliveries initiated by prelabor cesarean or induction of labor at preterm gestations(5, 10). These are often contrasted with all other preterm deliveries (i.e. 'spontaneous' preterm deliveries), or spontaneous preterm labor with intact membranes, and preterm premature rupture of membranes(10, 11, 17, 20, 21).

Subdividing preterm deliveries into subtypes can diminish statistical power because fewer women are present in each outcome category; however, this effect may be offset by improved specificity. Savitz et. al. empirically assessed whether the magnitude of associations of many well-known or "strongly suspected" risk factors differed statistically (P<.20) between indicated and spontaneous deliveries using data from the Pregnancy, Infection, and Nutrition (PIN) study (N=2319; 158 (55%) spontaneous, 128 (45%) indicated preterm deliveries).(10) The authors found that poverty index, clinic site, parity, bacterial vaginosis, and body mass index differed in the magnitude of their associations with spontaneous or medically indicated preterm births, while race, maternal age, marital status, education, prior preterm delivery, maternal height, and smoking had similar associations between the two preterm delivery subtypes. The authors concluded that the choice of whether to "lump" or "split" should be made based on the study question, balancing the loss of statistical power from excluding medically indicated births with the gain in precision from having a more etiologically homogeneous sample.

Substantial heterogeneity exists even within these preterm delivery subtypes. Preterm deliveries may be initiated for many maternal or fetal indications. Common maternal indications for preterm birth include hypertensive disorders, antepartum hemorrhage, placenta previa and diabetes(22). Common fetal indications include

intrauterine growth restriction, oligohydramnios, malformations, or fetal distress(22). Causes of spontaneous preterm deliveries may be equally varied, with infection, stress, uterine distension, nutritional factors, environmental exposures, and multiple vascular placental problems operating along separate pathways or in tandem(6-8, 23). Some avenues of research have focused on specific pregnancy complications (e.g. pre-eclampsia, placental abruption, intrauterine growth restriction) and have considered those cases that culminate in preterm delivery as more severe or as resulting from different causes(24, 25). Pregnancy complications may inform research into etiologic pathways that lead to preterm delivery through subclinical manifestations of similar pathologic processes.

In 2005, the March of Dimes published a research agenda that identified four "major pathophysiologic pathways" to preterm delivery: infection/inflammation, maternal/fetal stress, abruption or decidual hemorrhage, and mechanical stretch(3). The most studied pathway to date has been infection/inflammation. Intrauterine infection causes a significant proportion of spontaneous PTD, particularly earlier deliveries. Bacteria ascend from the lower genital tract before or during pregnancy, infect the membranes, and initiate an inflammatory response culminating in preterm labor or preterm premature rupture of membranes(9). These infections, which often have no symptoms, must be identified through sampling of amniotic fluid, fetal cord blood, or delivered placental tissue that shows evidence of histologic chorioamnionitis (HCA)(19). Other types of inflammation may also predispose women to preterm delivery(26).

The "abruption or decidual hemorrhage" pathway has been less comprehensively studied. The overarching theme of this dissertation is to expand understanding of this

pathway by examining multiple sources of evidence of placental hemorrhage, including clinical evidence and evidence derived from placental pathology examinations using data from the prospective Pregnancy Outcomes and Community Health (POUCH) Study. Chapter 2 explores various hypothesized manifestations of placental hemorrhage and their relations with one another, maternal characteristics, and preterm delivery. Chapter 3 focuses on functional polymorphisms in three candidate genes that have previously been studied in relation to placental abruption, with a goal of investigating their relations with preterm deliveries that have evidence of placental hemorrhage. Chapter 4 delves into possible relations between evidence of placental hemorrhage and infection/inflammation identified prior to delivery or through histopathologic examination of delivered placental tissue. Chapter 5 provides a brief summary of the findings of chapters 2-4, and adds some thoughts about future directions.

1.2. Evidence of placental hemorrhage and preterm delivery

Placental abruption, defined as separation of the placenta from the uterus prior to delivery of the fetus, is a rare, potentially disastrous pregnancy complication diagnosed in approximately 1% of all pregnancies in the United States.(27) Placental abruption is a clinical diagnosis, typically made in response to symptoms such as pain, vaginal bleeding, and tetanic uterine contractions(28); however, these symptoms may have other causes, and they are not always present(29). Retroplacental hemorrhage at the separation site can result in a hematoma, a gross pathologic lesion of abruption. Some diagnoses are made (or confirmed) by visualization of a hematoma during an ultrasound scan(29-31); in clinical practice, however, whether a scan is performed depends on the level of clinical

suspicion or intensity of prenatal care. Sometimes a clot adherent to the placenta is used to diagnose or confirm abruption, but clots may be present due to normal intrapartum bleeding. After delivery, placental pathologists are sometimes consulted to corroborate a diagnosis. Pathologists typically look for evidence that a clot was present *prior to* delivery, including infarcted, compressed, or otherwise affected adjacent tissue(32, 33).

Epidemiologic studies of risk factors for placental abruption have not employed consistent case definitions (Table 1.1). Many studies have focused on clinical diagnosis without specifying diagnostic criteria, sometimes taking diagnoses entered on birth certificates, registries or hospital discharge databases(24, 27, 34-36). Other definitions have required combinations of signs and symptoms. These have included sonographic visualization of retroplacental hematoma(37, 38), vaginal bleeding after 20 weeks or at delivery(8, 39-42), uterine pain or tenderness(38, 40, 41, 43-45), and strong contractions or increased uterine tone(38, 40, 43-45). Some studies have required presence of an adherent retroplacental clot or placental histopathology findings in addition to other signs and symptoms(39, 41, 42, 45). At the extreme, a few studies have required cases to cross a certain threshold of severity as evidenced by grade(46-48), necessity of immediate delivery(49), or fetal demise(50). Information on diagnoses from birth certificates or hospital discharge codes may not be uniform, both because practitioners may differ in their propensity to apply the diagnostic label and because women with other high-risk conditions or complications may receive more careful chart review and be more likely to have abruptions documented in administrative records(51). Thus, although they have the advantage of large samples, studies of abruption risk factors based purely on administrative or vital data could perpetuate inflated estimates of the risks associated with

Table 1.1. Placental abruption case definitions from selected epidemiologic studies

Author, Date	Placental abruption case definition
Williams 1992(52) Bartha 1996(53)	Not specified
Ananth 2001(35) Ananth 2006(24)	Diagnosis entered on birth certificate
Nurk 2004(36)	Birth registry
Casey 2005(54) Ananth 1996(34)	Perinatal database (hospital or regional)
Ananth 2005(27) El Kady 2004(55)	Hospital discharge codes (ICD-9 or ICD-10)
Misra 1999(56) Prochazka 2007(57)	Clinical diagnosis (usually by attending physician at delivery)
Ananth 2007(38) Nath 2007(44)	Clinical diagnosis based on signs and symptoms (painful vaginal bleeding, uterine pain, tenderness, hypertonicity); and/or retroplacental clot; and/or prenatal sonographic diagnosis; confirmed by chart review
Kramer 1997(40)	At least two of the following: 1) antepartum hemorrhage after 20 weeks' gestation, 2) uterine pain or tenderness, 3) fetal distress or death, and 4) retroplacental clot.
Mousa 2000(58)	Placental abruption requiring immediate delivery
Hira 2002(48) Agorastos 2002(46) Anteby 2004(47)	Abruption, grade 2 or 3
Parle-McDermott 2005(59)	"Severe" placental abruption; defined as retroplacental clot and/or accidental hemorrhage with associated clinical signs of abruption and/or a statement in the case records that the patient was a definite case of abruption placentae.
Naidu 2007(50)	Severe placental abruption resulting in stillbirth
Karakantza 2008(37)	Documented by routine second or third trimester ultrasound or in ultrasound performed in response to bleeding and/or abdominal pain
Jaaskelainen 2008(39)	Clinical diagnosis plus retroplacental clot, vaginal bleeding, uterine tenderness, increased baseline tone by external monitor, or fetal distress or death
Larciprete 2007(41)	Clinical diagnosis based on antepartum uterine tenderness and vaginal bleeding and confirmed by inspection of the placenta at delivery

Table 1.1 (cont'd).

Dizon-Townsend 2005(60)	Clinical suspicion supported by written documentation (excessive bleeding, treatment with blood products, description of delivery and placenta, and/or confirmation by pathologic exam
Wiener-Megnani 1998(42)	Profuse vaginal bleeding not from placenta previa appearing during the third trimester of pregnancy and the clinical observation of the placenta
Zhang 2007(45)	Clinical findings of vaginal bleeding without placenta previa, abdominal pain, uterine tenderness, contractions; also placental blood clots compressing adjacent villi
Salafia 1995(61)	Clinical: antepartum vaginal bleeding judged clinically to be the primary complication leading to preterm delivery Pathological: gross retroplacental hematoma with subjacent placental infarct or villous infarct with microscopic evidence of basal plate destruction or deformation
McElrath 2008(8)	Presentation with a significant amount of vaginal bleeding (documented in medical record or postpartum hematocrit <24%) and a clinical diagnosis of placental abruption in the absence of cervical change, among deliveries <28 weeks

well-known risk factors; on the other hand, such studies could underestimate risks associated with less publicized or poorly documented risk factors.

Several lines of evidence implicate placental hemorrhage in the pathophysiology of some preterm deliveries (PTD). Diagnosis of placental abruption is more commonly made at preterm than term gestations(24). Early pregnancy vaginal bleeding has been associated with both preterm delivery (PTD)(20, 21, 62) and placental abruption.(29, 31) Hemosiderin –suggesting old intrauterine bleeding – was found more often among very preterm (<32 weeks) placentas than term placentas in one study(33). Intrauterine (subchorionic or retroplacental) hematomas identified in early pregnancy by ultrasound scans also marked women at high risk of preterm delivery and placental abruption(63). Elevated maternal serum alpha-fetoprotein (MSAFP), a possible biomarker of disturbed

uteroplacental vascular integrity, has been consistently associated with increased PTD risk(52, 64-68) and more strongly associated with abruption risk.(52, 66, 68-72)

We hypothesized that clinically diagnosed placental abruption may be the tip of an epidemiologic iceberg of placental hemorrhage. Clinically evident placental abruption may only represent a portion of the clinically relevant involvement of bleeding in delivery timing, because subclinical hemorrhage may also contribute to risk of PTD without reaching a threshold necessary for clinical detection. Clinical diagnosis of placental abruption is likely to be related to severity of signs and symptoms, presence of risk factors, and intensity of prenatal care. Subclinical hemorrhage that predisposes a woman to PTD could plausibly share similar risk factors with clinically diagnosed abruption.

1.3. Polymorphisms in genes involved in vascular function in relation to evidence of placental hemorrhage and preterm delivery

Because placental abruption involves an acute vascular disruption and has been consistently linked to high blood pressure(73-76), underlying vascular causes have been investigated in its etiology. At least two pathophysiologic mechanisms originating with vascular dysfunction have been implicated in placental abruption risk. Normal pregnancy alters hemostatis, shifting the balance to a relatively hypercoagulable state(77). Inherited thrombophilias may exacerbate this shift and predispose women to develop decidual artery thrombosis, which may lead to necrosis and venous hemorrhage, ultimately resulting in a retroplacental hematoma(78). The renin-angiotensin- system regulates fluid balance and blood pressure. An increase in angiotensin may result in blood vessel

constriction and sodium and water retention, thereby causing blood pressure to increase,(79-81) and increased blood pressure may contribute to blood vessel rupture in the maternal-fetal interface.

Recurrence risk for placental abruption is very high (~10-15%, reviewed by Ananth, Savitz and Williams(73)), suggesting that factors specific to the mother – perhaps genetic factors – may contribute to abruption risk. Candidate genes in vascular pathways, including those involved in hemostasis and hemodynamics, have been identified. Polymorphisms that have been implicated in a thrombophilic predisposition include methylenetetrahydrofolate reductase (*MTHFR*) C677T, MTHFR A1298C, Factor 2 (prothrombin) G20210A, and Factor V (*F5*) G1691A (i.e. the Leiden variant, abbreviated FVL). The C704T promoter polymorphism (also known as Met235Thr) in the angiotensinogen (*AGT*) gene, which codes for an angiotensin precursor peptide, has been associated with increased risk of hypertension in some studies, although results have been inconsistent(82).

Table 1.2 shows studies reporting on associations between selected polymorphisms in *MTHFR*, *F5*, and *AGT* and placental abruption, adapted from a recent HuGE review and metaanalysis by Zdoukoupoulos and Zintzaras(83). For MTHFR polymorphisms, the metaanalysis provided little evidence that dominant models for MTHFR(677) (based on 9 studies, pooled fixed effects OR=1.24 (95% CI 0.83, 1.85)) or MTHFR(1298) (based on 3 studies, pooled fixed effects OR=1.33 (95% CI 0.97, 1.83)) conferred increased risk of abruption. For FVL, the metaanalysis identified significantly increased abruption risk based on 10 studies, with a pooled random effects odds ratio of 3.42 (95% CI 1.62, 3.41). For the AGT C704T variant, only two studies of were included

in the meta-analysis; the larger (62 cases, 240 controls) found increased abruption risk for the dominant model (OR=4.35), while the smaller (50 cases, 50 controls) found a non-significant OR of 0.5.

Several published studies describing associations between these four polymorphisms and placental abruption were not included in the Zdoukopoulos and Zintzaras review and metaanalysis(36, 37, 41, 57, 84, 85) (see Table 1.2). Although no table of excluded studies was presented, reasons that studies were not included in the meta-analysis may be that they were been published too late(57), were cohort (rather than case-control) studies(36, 37, 84), were not published in English(85), and had incomplete descriptions of genotype frequencies(41)). Results for the FVL dominant models from the excluded studies that reported odds ratios were generally consistent with the studies included in the meta-analysis (OR range 3.0 to 9.1)(37, 57, 84). The excluded studies reporting on the two MTHFR polymorphisms did not report results of a dominant model(36, 37, 41, 57).

The Zdoukopoulos and Zintzaras metaanalysis did not report pooled results for recessive models. However, for the two MTHFR variants, the dominant model may not be the biologically relevant model. Among ten studies that reported an odds ratio for the MTHFR(677) TT allele – either versus CT/TT or versus CC – results have been inconsistent, having odds ratios ranging from 0.58 to 2.65(36-38, 41, 50, 57, 59, 86-88). Among five studies that reported an odds ratio for the MTHFR(1298) CC genotype, odds ratios have ranged from 0 to 4.36(36, 38, 41, 59, 89).

Table 1.2. Studies reporting on associations between selected vascular function gene polymorphisms and placental abruption, adapted from Zdoukoupoulos and Zintzaras

First Author, Year	Study Area, Ethnicity	Study Population	Placental Abruption Diagnostic Criteria	Gene (Poly-morphism)	Genotype Distribution mtmt/mtwt/wtwt	Comparison	OR (95% CI)
Factor V Leiden, included in Zdoukoupoulos and Zintzaras meta-analysis(90)							
Wiener-Megnagi, 1998(42)	Israel, mixed	27 cases, (29.5 ± 5.4) yrs	Vaginal bleeding plus clinical observation of the placenta	F5 (Arg506Gln)	Cases: 3/5/19 Controls: 0/1/28	GlnGln vs. GlnArg/ArgArg GlnGln/GlnArg vs. ArgArg *Gln vs. *Arg	na 11.8 (1.36-102) na
Kupferminc, 1999(88)	Israel, Jews	20 cases 110 controls, matched for age, ethnicity	Clinical criteria	F5 (Arg506Gln)	Cases: -/5/15 Controls: 0/7/103	GlnGln vs. GlnArg/ArgArg GlnGln/GlnArg vs. ArgArg GlnGln vs. GlnArg *Gln vs. *Arg	na 4.9 (1.37-17.4) na N/A
Alfirevic, 2001(91)	UK, mixed	23 cases 44 controls, matched for age, parity, gestation	N/A	F5 (Arg506Gln)	Cases: 0/0/23 Controls: 0/3/41	GlnGln vs. GlnArg/ArgArg GlnGln/GlnArg vs. ArgArg GlnGln vs. GlnArg *Gln vs. *Arg	na na na na
Agorastos, 2002(46)	Greece, Greeks	7 cases 100 controls, matched for ethnicity	Clinical criteria plus clinical observation of the placenta	F5 (Arg506Gln)	Cases: 0/3/4 Controls: 0/4/96	GlnGln vs. GlnArg/ArgArg	na

Table 1.2 (cont'd).

First Author, Year	Study Area, Ethnicity	Study Population	Placental Abruption Diagnostic Criteria	Gene (Poly-morphism)	Genotype Distribution mtmt/mtwt/wtwt	Comparison	OR (95% CI)
Hira, 2002(48)	South Africa, blacks	100 cases 217 controls	Clinical criteria	F5 (Arg506Gln)	Cases: 0/0/100 Controls: 0/-/217	GlnGln vs. GlnArg/ArgArg GlnGln/GlnArg vs. ArgArg GlnGln vs. GlnArg *Gln vs. *Arg	na na na na
Facchinetti, 2003(92)	Italy, whites	50 cases, (31.7 ± 5.9) yrs 100 controls matched for age, parity, ethnicity	Clinical criteria plus histological examination of the placenta	F5 (Arg506Gln)	Cases: 0/11/39 Controls: 0/3/97	GlnGln vs. GlnArg/ArgArg GlnGln/GlnArg vs. ArgArg GlnGln vs. GlnArg *Gln vs. *Arg	na 9.12 (2.18, 31.7) na 8.11 (2.20, 29.80)
Prochazka, 2003(93)	Sweden, whites	102 cases (30.1 [SD 5.6]) yrs 2371 controls	Clinical criteria plus clinical examination of the placenta	F5 (Arg506Gln)	Cases: -/16/86 Controls: -/255/2116	GlnGln vs. GlnArg/ArgArg GlnGln/GlnArg vs. ArgArg GlnGln vs. GlnArg *Gln vs. *Arg	N/A 1.5 (0.9-2.7) N/A N/A
Jaaskelainen, 2004(86)	Finland, whites	116 cases, (30 [28.9-31.1]) yrs 112 controls	Clinical criteria plus clinical observation or histological examination of the placenta	F5 (Arg506Gln)	Cases: 0/3/113 Controls: 0/4/108	GlnGln vs. GlnArg/ArgArg GlnGln/GlnArg vs. ArgArg GlnGln vs. GlnArg *Gln vs. *Arg	na 0.7 (0.15-3.28) na 0.7 (0.15-3.25)

Table 1.2 (cont'd).

First Author, Year	Study Area, Ethnicity	Study Population	Placental Abruption Diagnostic Criteria	Gene (Polymorphism)	Genotype Distribution mtmt/mtwt/wtwt	Comparison	OR (95% CI)
Dizon-Townson, 2005(60)	USA, mixed	31 cases 4436 controls	Clinical criteria or histological examination of the placenta	F5 (Arg506Gln)	Cases: 0/0/31 Controls: 0/121/4315	GlnGln vs. GlnArg/ArgArg GlnGln/GlnArg vs. ArgArg GlnGln vs. GlnArg *Gln vs. *Arg	na na na na
Jarvenpaa, 2006(87)	Finland, whites	9 cases 111 controls	Clinical criteria	F5 (Arg506Gln)	Cases: 0/2/7 Controls: 0/2/109	GlnGln vs. GlnArg/ArgArg GlnGln/GlnArg vs. ArgArg GlnGln vs. GlnArg *Gln vs. *Arg	na 15.57 (1.9-127) na 13.75 (1.81-104)
Factor V Leiden, not included in Zdoukoupoulos and Zintzaras Meta-analysis							
Kocher, 2007(84)	USA, whites (cohort)	22 cases, 66 controls matched on age, parity	Clinical criteria	F5 (Arg506Gln)	Cases: -/2/20 Controls: -/1/65	GlnGln/GlnArg vs. ArgArg	6.50 (0.56-75.46)
Koleva, 2005(85)	Bulgaria (Article in Bulgarian)	14 cases, 103 controls	Unknown	F5 (Arg506Gln)	Cases: -/6/8 Control: -/6/97	GlnGln/GlnArg vs. ArgArg	N/A
Larciprete, 2007(41)	Italy	16 cases 176 controls (no severe PE, HELLP, GH, FGR, IUFD, or DIC)	Clinical criteria plus clinical examination of the placenta	F5 (Arg506Gln)	Cases: 0/?/? Controls: ? Can't abstract from information given in article	GlnGln vs. GlnArg/ArgArg	0

Table 1.2 (cont'd).

First Author, Year	Study Area, Ethnicity	Study Population	Placental Abruption Diagnostic Criteria	Gene (Poly-morphism)	Genotype Distribution mtmt/mtwt/wtwt	Comparison	OR (95% CI)
Prochazka, 2007(57)	Czech Republic (cohort)	142 cases, 196 controls	Clinical criteria plus clinical examination of the placenta	F5 (Arg506Gln)	Cases: -/20/122 Controls: -/10/186	GlnGln/GlnArg vs. ArgArg	3.0 (1.4-6.7)
Karakantza, 2008(37)	Greece, Greeks (cohort)	15 cases, 377 controls	Ultrasound-confirmed	F5 (Arg506Gln)	Cases: 0/3/12 Controls: 0/10/367	GlnGln/GlnArg vs. ArgArg	9.1 (2.2-37)
MTHFR(677) included in meta-analysis							
Kupferminc, 1999(88)	Israel, Jews	20 cases 110 controls, matched for age, ethnicity	Clinical criteria	MTHFR (C677T)	Cases: 3/-/17 Controls: 9/-/17	TT vs. TC/CC TT/TC vs. CC TT vs. TC *T vs *C	1.98 (0.48-8.06) N/A N/A N/A
Alfirevic, 2001(91)	UK, mixed	23 cases 44 controls, matched for age, parity, gestation	N/A	MTHFR (C677T)	Cases : 0/-/23 Controls : 2/-/42	TT vs. TC/CC TT/TC vs. CC TT vs. TC *T vs. *C	na N/A na N/A
Gebhardt, 2001(89)	South Africa, blacks	18 cases 114 controls	Clinical criteria plus clinical observation of the placenta	MTHFR (C677T)	Cases: 0/5/13 Controls: 2/30/82	TT vs. TC/CC TT/TC vs. CC	Na 0.99 (0.28-3.30)
Hira, 2002(48)	South Africa, blacks	100 cases 217 controls	Clinical criteria	MTHFR (C677T)	Cases: 0/13/87 Controls: 2/22/193	TT vs. TC/CC TT/TC vs. CC TT vs. TC *T vs. *C	Na 1.2 (0.58-2.47) Na 1.0 (0.54-2.17)

Table 1.2 (cont'd).

First Author, Year	Study Area, Ethnicity	Study Population	Placental Abruption Diagnostic Criteria	Gene (Polymorphism)	Genotype Distribution mtmt/mtwt/wtwt	Comparison	OR (95% CI)
Parle-McDermott, 2005(59)	Ireland, whites	62 cases	Clinical criteria or clinical observation of the placenta or a statement in the case records that the patient was a definitive case of placental abruption	MTHFR (C677T)	Cases: 5/31/26 Controls: 22/80/80	TT vs. TC/CC TT/TC vs. CC TT vs. TC *T vs. *C	0.64 (0.23-1.76) 1.0 (0.6-1.94) 0.58 (0.2-1.68) 0.96 (0.63-1.44)
Jaaskelainen, 2006(43)	Finland, whites	117 cases, (30 [28.9-31.1]) yrs 112 controls	Clinical criteria plus clinical observation or histological examination of the placenta	MTHFR (C677T)	Cases: 6/36/75 Controls: 2/38/298	TT vs. TC/CC TT/TC vs. CC TT vs. TC *T vs. *C	0.96 (0.30-3.05) 0.74 (0.43-1.27) 1.16 (0.34-3.93) 0.81 (0.52-1.26)
Naidu, 2006(50)	South Africa, blacks	155 cases, (27 [13-44]) 338 controls	Clinical criteria plus sonographic diagnosis plus clinical observation of the placenta	MTHFR (C677T)	Cases : 1/21/133 Controls : 2/38/298	TT vs. TC/CC TT/TC vs. CC TT vs. TC *T vs. *C	1.09 (0.09-12.12) 1.23 (0.70-2.15) 0.90 (0.07, 10.57) 1.20 (0.71-2.04)
Jarvenpaa, 2006(87)	Finland, whites	9 cases 111 controls	Clinical criteria	MTHFR (C677T)	Cases : 1/-/8 Controls : 5/-/106	TT vs. TC/CC TT/TC vs. CC TT vs. TC *T vs. *C	2.65 (0.27-25.5) N/A N/A N/A
Ananth, 2007(38)	USA, mixed	196 cases 189 controls Matched for parity, race/ethnicity	Clinical criteria or clinical observation of the placenta or sonographic diagnosis	MTHFR (C677T)	Cases: 26/69/100 Controls: 33/69/87	TT vs. TC/CC TT/TC vs. CC TT vs. TC *T vs. *C	0.72 (0.41-1.27) 0.81 (0.54-1.21) 0.78 (0.42-1.45) 0.81 (0.60-1.10)

Table 1.2 (cont'd).

First Author, Year	Study Area, Ethnicity	Study Population	Placental Abruption Diagnostic Criteria	Gene (Poly-morphism)	Genotype Distribution mtmt/mtwt/wtwt	Comparison	OR (95% CI)
MTHFR(677) not included in Zdoukoupoulous and Zintzaras meta-analysis							
Karakantza 2008(37)	Greece, Greeks	15 cases, 377 controls	Ultrasound-confirmed	MTHFR (C677T)	Cases: 6/9/- Controls: 36/260/-	TT vs. TC/CC	1.72 (0.93-3.17)
Larciprete 2007(41)	Italy	16 cases 176 controls (no severe PE, HELLP, GH, FGR, IUFD, or DIC)	Clinical criteria plus clinical examination of the placenta	MTHFR (C677T)	Cases: 3/-/13 Controls: 60/-/116	TT vs. TC/CC	0.58 (0.39-0.71)
Nurk 2005(36)	Norway (cohort)	74 cases 14484 controls	Birth registry	MTHFR (C677T)	Cases: 33/26/15 Controls: 7165/6037/1282	TC vs. CC TT vs. CC	1.0 (0.6-1.6) 2.6 (1.4-4.8)
Prochazka 2007(57)	Czech Republic	142 cases, 196 controls	Clinical criteria plus clinical examination of the placenta	MTHFR (C677T)	Cases: 8/-/134 Controls: 7/-/189	TT vs. TC/CC?	1.6 (0.6-4.7)
MTHFR(1298) included in Zdoukoupoulos and Zintzaras meta-analysis							
Gebhardt, 2001(89)	South Africa, blacks	18 cases 114 controls	Clinical criteria plus clinical observation of the placenta	MTHFR (A1298C)	Cases: 3/9/6 Controls: 5/39/70	CC vs. CA/AA CC/CA vs. AA CC vs. CA *C vs. *A	4.36 (0.94-20.1) 3.18 (1.01-10.37) 2.60 (0.52-12.9) 2.61 (1.18-5.77)

Table 1.2 (cont'd).

First Author, Year	Study Area, Ethnicity	Study Population	Placental Abruption Diagnostic Criteria	Gene (Poly-morphism)	Genotype Distribution mtmt/mtwt/wtwt	Comparison	OR (95% CI)
Parle-McDermott, 2005(59)	Ireland, whites	62 cases	Clinical criteria or clinical observation of the placenta or a statement in the case records that the patient was a definitive case of placental abruption	MTHFR (A1298C)	Cases: 6/31/25 Controls: 18/75/91	CC vs. CA/AA CC/CA vs. AA CC vs. CA *C vs. *A	0.98 (0.37-2.61) 1.45 (0.81-2.6) 0.80 (0.29-2.22) 1.23 (0.81-1.86)
Ananth, 2007(38)	USA, mixed	196 cases 189 controls Matched for parity, race/ethnicity	Clinical criteria or clinical observation of the placenta or sonographic diagnosis	MTHFR (A1298C)	Cases: 16/62/187 Controls: 7/64/118	CC vs. CA/AA CC/CA vs. AA CC vs. CA *C vs. *A	2.32 (0.93-5.78) 1.10 (0.73-1.67) 2.36 (0.91-6.12) 1.22 (0.86-1.71)
MTHFR(1298) not included in Zdoukoupoulos and Zintzaras meta-analysis							
Larciprete 2007(41)	Italy	16 cases 176 controls (no severe PE, HELLP, GH, FGR, IUFD, or DIC)	Clinical criteria plus clinical examination of the placenta	MTHFR (A1298C)	Cases: 0/-/16 Controls: ? Not clear from information given in paper	CC vs. CA/AA (suspect)	0
Nurk 2005(36)	Norway (cohort)	74 cases 14484 controls	Birth registry	MTHFR (A1298C)	Cases: 35/32/6 Controls: 6607/6342/1525	CC vs. AA CA vs. AA	1.0 (0.6-1.6) 0.7 (0.3-1.7)

Table 1.2 (cont'd).

First Author, Year	Study Area, Ethnicity	Study Population	Placental Abruption Diagnostic Criteria	Gene (Poly-morphism)	Genotype Distribution mtmt/mtwt/wtwt	Comparison	OR (95% CI)
AGT(1298) included in Zdoukoupoulos and Zintzaras meta-analysis							
Hillermann, 2005(94)	South Africa, blacks	50 cases, (28 [16-42]) yrs 50 controls	Clinical criteria plus clinical observation of the placenta	AGT(Met235Thr)	Cases: 32/12/4 Controls: 31/12/2	ThrThr vs. ThrMet/MetMet ThrThr/ThrMet vs. MetMet ThrThr vs. ThrMet *Thr vs. *Met	0.9 (0.37-2.16) 0.5 (0.09-2.94) 1.03 (0.40-2.64) 0.82 (0.39-1.70)
Zhang, 2007(45)	USA, mixed	62 cases, (26.65 ± 6.6)	Clinical criteria plus histological examination of the placenta	AGT(Met235Thr)	Cases: 26/27/9 Controls: 43/95/102	ThrThr vs. ThrMet/MetMet ThrThr/ThrMet vs. MetMet ThrThr vs. ThrMet *Thr vs. *Met	3.30 (1.81-6.04) 4.35 (2.05-9.22) 2.12 (1.11-4.06) 2.90 (1.92-4.36)
AGT(1298) not included in Zdoukoupoulos and Zintzaras meta-analysis							
(None identified)							

N/A Not applicable. Na, not available

Fewer studies have been published on the associations between vascular function genotypes and preterm delivery, either overall or by subtypes. Valdez-Velazquez found a two-fold increased risk of preterm premature rupture of membranes with the homozygous or heterozygous methionine genotypes (normally considered the major allele) vs. the homozygous threonine genotype of the AGT polymorphism in Mexican women compared with normal pregnancy community controls(95). Four studies specifically reported on associations between FVL and preterm delivery, and none found an association(84, 96-98). Of five studies identified that reported on associations between MTHFR(677) and PTD(96-100), two found non-significant elevated risk with genotypes with at least one copy of the T allele(98, 100). Finally, one study reported a null result for MTHFR(1298) and PTD(96).

Few prior studies have studied genetic polymorphisms with respect to specific placental lesions. Ariel and colleagues examined fetal and maternal vascular lesions in relation to fetal inherited thrombophilias (FVL, prothrombin, or homozygous MTHFR polymorphisms) in a series of 64 placentas from pregnancies complicated by preeclampsia, placental abruption, or intrauterine growth restriction, and found no association(101). Many and colleagues investigated 68 placentas from complicated singleton pregnancies (i.e. those with severe preeclampsia, intrauterine growth retardation, stillbirth or placental abruption). They compared 12 placental findings between the 32 from women with at least one thrombophilia (FVL, MTHFR, or prothrombin mutation or deficiency in proteins S, C, or antithrombin III) and the 36 having no thrombophilia(102). Only four placental findings differed significantly between the thrombophilia and non-thrombophilia groups: the thrombophilia group had

lower placental weight, more frequently had single or multiple villous infarcts, and more often had fibrinoid necrosis. Mousa et. al. used a similar design to study placentas from 79 pregnancies complicated by severe pre-eclampsia/eclampsia, placental abruption, intrauterine growth restriction, or stillbirth; 43 women had at least one thrombophilic abnormality (antithrombin III, protein C, protein S, activated protein C resistance, anticardiolipin antibodies, lupus anticoagulant, fasting plasma homocysteine and specific mutations to methylenetetrahydrofolate reductase C677T, G20210A prothrombin gene and factor V Leiden)(58). The majority of women in both groups had abnormal histopathology, but no specific lesion pattern correlated with thrombophilia. These studies have suffered from two important limitations. First, these studies considered several inherited and acquired thrombophilias as a group even though their effects may be heterogeneous. Second, none of these studies included normal pregnancy controls. There may be multiple exposures (known thrombophilias, as-yet-identified thrombophilias, or other abnormalities) that cause the placental abnormalities evident among placentas from pregnancies having severe complications, and it may be informative to compare thrombophilia status between pregnancies with complications to those with normal outcomes.

1.4. Evidence of placental hemorrhage in relation to inflammation and infection

It is well-accepted from many lines of evidence that ascending infection is causally related to some preterm deliveries, especially early preterm deliveries(11). Subclinical infections in gestational tissues are believed to trigger an inflammatory cytokine cascade that causes uterine contractions resulting in either spontaneous preterm

labor (SPTL) or membrane degradation resulting in premature rupture of membranes (PROM). Another pathway to spontaneous preterm delivery, decidual hemorrhage, has been described by Lockwood(103). This "bleeding pathway" is sometimes conflated with the clinical circumstance of placental abruption(104); however it may be one mechanism of several leading to clinically diagnosed placental abruption. Limited epidemiologic evidence has linked placental abruption, decidual hemorrhage, or other gestational uterine bleeding to infection or other inflammatory processes. Studies that have investigated associations between evidence of placental hemorrhage and evidence of intrauterine infection are summarized in Table 1.3.

An analysis of data from the National Maternal and Infant Health Survey found that, at preterm gestations, abruption diagnoses are associated with diagnoses of acute inflammation-related conditions such as preterm PROM and intrauterine infections(24). Several other articles have reported statistical associations between diagnosed chorioamnionitis or intrapartum fever and abruption(27, 29, 105). Moreover, abruption is recognized (by the American College of Obstetricians and Gynecologists among others) as a possible complication of preterm PROM(73, 105-107). If the PROM has an inflammation-related etiology (e.g., Ananth et. al. grouped PROM with non-PROM intrauterine infections as "acute inflammation"-associated conditions(24)), then any resulting abruption could be indirectly attributable to inflammation (perhaps via subsequent reduced uterine volume and attachment surface area(105)) or more directly attributable if the inflammatory process causes placental vessel friability resulting in decidual hemorrhage(108).

Table 1.3. Studies comparing evidence of placental hemorrhage with clinical or histologic evidence of infection

Author, Date	Design	Sample	Evidence of Infection	Evidence of Bleeding	Race/ethnic composition	Results
French 1999(108)	Cohort	Secondary analysis of screening and treatment trial for common vaginal infections in Denver, CO	CCA: intrapartum antibiotic for suspected or presumed chorioamnionitis with ≥2 of: fever, leukocytosis, maternal tachycardia, fetal tachycardia, uterine tenderness, or foul-smelling amniotic fluid.	First, second, and third trimester vaginal bleeding, ascertained during prenatal care visits.	34% white or Asian, 50% Hispanic, 3% Native American, 14% black	First trimester, RR=2.5 (1.9, 15.4); Second trimester: RR=2.2 (0.9, 5.5); Third trimester: RR=3.4 (1.4, 8.3)
De Felice 1997(109)	Cohort	Siena, Italy. Sample includes 33 women with first or second trimester bleeding and 66 women with no bleeding	HCA and CCA. HCA defined as at least 10 PMNLs in at least 10 non-adjacent fields (sampled membranes at rupture site, 3 samples of cord, and 3 full thickness placental samples, or decidual necrosis with a few cord PMNLs. CCA defined as PROM >48 hours, or uterine tenderness with white blood cell count >12,500, or fever >38C or erythrocyte sedimentation rate >40 mm in first hour	First or second trimester vaginal bleeding	Not stated.	HCA: 54.5% with bleeding vs. 20.6% without bleeding, p<.001. CCA: 15.1% vs. 17.4%, p=1.0.

Table 1.3 (cont'd)

Author, Date	Design	Sample	Evidence of Infection	Evidence of Bleeding	Race/ethnic composition	Results
Boggess 2006(110)	Retro-spective cohort	Secondary analysis of 660 women from the Oral Conditions and Pregnancy Study.	Cord blood IgM for oral pathogens	First and second trimester vaginal bleeding ascertained by questionnaire within 48 hours of delivery.	50% white	Vaginal bleeding in first, second, or both trimesters associated with IgM (RRs 1.7, 2.1, and 1.6, respectively) after adjusting for race, prior PTD, 2 or more previous abortions, bacterial vaginosis, and enrollment weight.
Salafia 1995(61)	Case series	Placents from deliveries <32 weeks, N=253	Umbilical vasculitis	Hemosiderin in placental tissue	Not stated	Inverse association between hemosiderin and neutrophil infiltration of umbilical cord.
Ananth 2006(24)	Retro-spective cohort	National Center for Health Statistics linked birth/fetal death certificates 1995-2002, N=30,378,902	Fever with and without PROM at term and preterm gestations	Placental abruption: checked on birth certificate	80% white	RR range 1.55 to 1.92, all significant. Population attributable fractions: 0.7% term, 2.4% preterm.

Table 1.3 (cont'd)

Author, Date	Design	Sample	Evidence of Infection	Evidence of Bleeding	Race/ethnic composition	Results
Ananth 2004(105)	Retro-spective cohort	National Maternal and Infant Health Study, 1988, N=11,777	CCA: fever plus uterine tenderness, foul discharge or positive amniotic fluid culture; also used HCA when available, but no information on proportion of each (incidence 4.3%)	Placental abruption: complete or partial separation of a normally implanted placenta before delivery, usually accompanied by abdominal pain and vaginal bleeding (data from vital records, maternal and hospital interview and prenatal provider questionnaire)	46% black (unweighted)	Unadjusted: 6.06 (2.40–15.35). Adjusted: 9.71 (3.23–29.17)
Tikkanen 2006(29)	Case control	Single hospital, Helsinki Finland, 198 cases, 396 controls	Clinical chorioamnionitis (maternal fever, uterine tenderness, foul AF odor, increased maternal and fetal heart rate, increased WBC, increased heart rate)	Placental abruption: Initially ID'd by ICD-9 code, then determined by clinical judgment and US findings, routinely done at 10-13 and 18-20 wks, confirmed by rp adherent hematoma, couvelaire uterus, or blood clot at c-section.	Finns	OR=3.3 (1.1, 10.2)
Saftlas 1991(111)	Cohort	National Hospital Discharge Survey, 1979-1987	chorioamnionitis by ICD-9-CM codes	Placental abruption by ICD-9-CM codes	21% black	RR=2.5 (1.6, 3.9)
Kramer 1997(40)	Cohort	Single hospital, Montreal, N=36875	severe leukocytic infiltration of placenta, membranes, or cord, 1.8% prevalence.	Placental abruption, required two of four criteria: 1) antepartum hemorrhage after 20 weeks' gestation, 2) uterine pain or tenderness, 3) fetal distress or death, and 4) retroplacental clot.	Text states that sample is ethnically diverse, but no details are given.	adjusted OR=2.61 (1.65, 4.12)

Table 1.3 (cont'd)

Author, Date	Design	Sample	Evidence of Infection	Evidence of Bleeding	Race/ethnic composition	Results
Naeye 1981(112)	Cohort	National Collaborative Perinatal Project, N=27,695	deciduo-chorioamnionitis, defined as acute inflammation at many sites in the deciduas and throughout the chorionic plate of the placenta, 12% prevalence:	Placental abruption: antepartum hemorrhage with retroplacental clot at delivery, 2% prevalence	Not stated	RR=2.0 calculated from data
Ananth 2006(31)	Cohort	National Collaborative Perinatal Project, N=46,364	Acute lesions included the following: decidual necrosis, and neutrophil infiltration in the amnion, chorion, placental surface, and umbilical vein	Placental abruption: Determined by attending at delivery according to clinical criteria	47.4% AA, 44.7% caucasian, 7.9% other	Unadjusted: Decidual Necrosis, OR=1.2 (1.0, 1.4). Neutrophil infiltration: of amnion OR=0.8 (0.6, 1.2); of chorion, OR=1.6 (1.3, 2.1); of placental surface, OR=1.2 (0.8, 1.7); of umbilical vein: OR=0.9 (0.6, 1.1).
Woods 1986(113)	Case-control	South Africa, 30 cases, 30 controls matched on gestational age.	Blanc's criteria for maternal and fetal inflammatory response	Severe placental abruption, accompanied by shock and fetal distress or demise	Not stated.	No association.

Table 1.3 (cont'd)

Author, Date	Design	Sample	Evidence of Infection	Evidence of Bleeding	Race/ethnic composition	Results
Nath 2007(44)	Case-control	Two tertiary, level III, regional perinatal centers). 170 cases, 176 controls. All but 24 preterm births had abruption, and all but 53 term births were non-abruption. Matched on race and parity.	inflammatory infiltrates of neutrophils at 2 or more sites on the chorionic plate and extraplacental membranes. Degree: Mild chorioamnionitis = few scattered (5-10 per high-powered field) neutrophils in the subchorionic space and adjacent chorion; moderate = many (11-30 per high powered field) neutrophils in the lower half of the chorionic plate; and severe = dense infiltrates of neutrophils (more than 30 per high powered field) throughout the chorionic plate into the amnion.	Painful vaginal bleeding or hemorrhage, uterine pain or tenderness, uterine hypertonicity, retroplacental clot, or hematoma on the placental surface or on the basis of prenatal sonographic diagnosis.	Approximately equal numbers of black, white, and hispanic women.	Preterm: Any vs. none 3.6 (1.7 to 10.5). Term: Any vs. None 2.8 (1.3 to 6.1)
Rana 1999(114)	Case-control	Nehru hospital, Chandigarh, India. 50 cases, 50 controls	membrane rolls: neutrophils present at many sits in theextraplacental membranes and in the subchorionic plate.	Placental abruption: Heavy vaginal bleeding associated with pain in abdomen or back and uterine tenderness or irritability and presence of retroplacental clots and signs of disruption of underlying placental tissue at delivery.	Not stated. Most abruption cases low SES, no prenatal care	12/40 (30%) of cases and 8/35 (22.9%) of controls had HCA; p>.05.

Table 1.3 (cont'd)

Author, Date	Design	Sample	Evidence of Infection	Evidence of Bleeding	Race/ethnic composition	Results
Darby 1989(115)	Case-control	37 severe preterm placental abruption cases, 51 indicated preterm delivery controls.	HCA: neutrophils present at many sites in the extraplacental membranes and in the subchorionic plate.	Placental abruption: confirmed by adherent retroplacental clot with depresion of underlying cotyledons.	79 white and 9 black women; 15% of cases and 6% of controls were black.	adjusted (for smoking and indigent patient status) OR=7.11 (1.76-28.7)

A handful of studies have explored a possible connection between hemorrhage and infection using placental pathology examinations, with contradictory findings. In the early 1980s, using data from the National Collaborative Perinatal Project (NCPP), Naeye found an association between "deciduo-chorio-amnionitis" and placental abruption(112). A more recent report using NCCP data reported modest (RR range 1.2 – 1.8) associations between placental lesions indicative of acute or chronic inflammation and placental abruption(31). A case-control study conducted in South Africa examined placentas from 30 cases of severe placental abruption and 60 non-abruption gestational age-matched controls, and found no association with measures of fetal or maternal inflammatory response(113). A single-hospital cohort study representing 36,875 births from 1978 to 1989 had diagnostic placental examination data on all deliveries. The authors found an association between placental abruption and histologic chorioamnionitis, with an odds ratio of 2.50 (95% CI 1.58, 3.98) after adjusting for maternal age, marital status, smoking, pre-pregnancy hypertension, pregnancy-induced hypertension, prolonged rupture of membranes, fetal sex, and small for gestational age fetus.(40) Recently, in the New Jersey Placental Abruption Study, a case-control study of abruption etiology, Nath et. al. found that severe HCA (defined by the authors as >30 neutrophils per high power field) was more common among abruption cases than controls; this pattern was similar for term and preterm deliveries(44).

In a series of placentas delivered prior to 32 weeks, Salafia found that hemosiderin deposition, indicative of previous hemorrhage, was inversely associated with histologic evidence of acute ascending infection but positively associated with chronic inflammation in the basal plate(33). Using another gestational age-truncated sample,

McElrath et. al. performed a factor analysis of a wide range of dichotomized variables (i.e. maternal demographics, newborn characteristics, placental histopathology, and placental microbiology) using data from the Extremely Low Gestational Age Newborn (ELGAN) Study(8). From this analysis, which included only births occurring prior to 28 completed weeks gestation, the authors concluded that placental abruption belonged with preterm labor, preterm premature rupture of membranes, and cervical insufficiency in an infection/inflammation group, while preeclampsia and preterm birth for fetal indication belonged together in a group characterized by impaired placentation.

Although placenta is an important tissue to study for evidence of infection, some studies have linked infection and bleeding using other biologic samples. Much of the research relating infection to preterm birth has focused on microbes ascending from the vaginal tract. Another area of inquiry has related to observed associations between periodontal disease/oral pathogens and risk of preterm birth (recently reviewed by Boggess et. al (116)). One study explored the association between antepartum vaginal bleeding, umbilical cord serum IgM-positive for oral pathogens, and risk of preterm birth at less than 35 weeks(110). The authors found evidence for effect modification, in that pregnancies with antenatal vaginal bleeding and fetal exposure to oral pathogens had a much higher risk of preterm birth than those with either exposure alone. They speculated on two potential mechanisms to explain their findings. First, the bleeding may have been evidence of a "disruption of the maternal-fetal interface" that allowed oral pathogens already present in the maternal circulation into fetal contact, which initiated a fetal response leading to PTD. Second, a fetal inflammatory response to oral pathogens from

another exposure route may have caused decidual inflammation that led to vaginal bleeding.

A prospective cohort study found that first trimester bleeding was associated with *Trichomonas vaginalis* (TV), *Chlamydia trachomatis*, and bacterial vaginosis (BV); further, that study found that women with both bleeding and BV or TV had a higher risk of spontaneous preterm birth than women with bleeding or infection alone(108). The authors suggested two possible mechanistic reasons for this apparent interaction: either the vaginal infection progresses to deciduitis/endometritis and causes clinically recognized bleeding, or bleeding from the decidua or trophoblast facilitates microbial ascent, interferes with host defenses, and/or serves as a substrate for bacteria, supporting the advancement of infection, and further aggravating the course of pregnancy.

A study from the Perinatology Research Branch at NICHD approached the infection-bleeding link by studying amniotic fluid from 114 women who had idiopathic vaginal bleeding during pregnancy(117). The bleeding was confirmed to be of uterine origin, and not attributable to placenta previa or overt placental abruption. Sixteen (14%) of these women had positive amniotic fluid bacterial cultures; this subset had the worst outcomes. The women with positive cultures were more likely than those with negative cultures to have histologic chorioamnionitis, subsequent preterm premature rupture of membranes, and spontaneous PTD, with 77% delivering prior to 28 weeks compared with 27.5% of the women with bleeding but negative cultures. Importantly, the study did not include a comparison group of non-bleeding women. The authors concluded that vaginal bleeding in these cases was a symptom of an otherwise subclinical amniotic fluid infection; however, they did not consider alternative explanations, such as that the

bleeding preceded and provided a nutritive substrate for bacteria, or that bleeding was unrelated to the infection.

In evaluating the strength of evidence from studies attempting to link the bleeding and infection/inflammation pathways to preterm birth, careful consideration should be given to several methodologic issues. Studies from large administrative databases typically have adequate sample sizes of abruption cases; however they can provide no information on time-order, biomarkers, or pathology, and inferring mechanisms from such studies is difficult. Further, since all information on outcomes and most information on putative exposures are derived from the same administrative data sources, non-differential misclassification and shared method variance may result in biased estimates of the degree of association, therefore these studies can not be considered conclusive with respect to determining any causal link between infection and abruption.

For inferring presence of intrauterine infection, studies have used either clinical(24, 29, 105, 108) or histologic(31, 40, 44, 109, 112-115) determinations, which might result in different conclusions. Placental examination is the most objective way to quantify both infection and hemorrhage. Unfortunately, it is not possible to definitively establish time order after delivery has occurred. Moreover, studies differ in their placental pathology measures, with some using clinical – rather than research protocol – placental exams, and the pathologist's knowledge of the clinical picture may have influenced their diagnoses. Even among studies that have employed research protocols, criteria used to define histologic chorioamnionitis have varied considerably, as recently reviewed in the context of preterm delivery.(19) Many of the studies reviewed here have been limited by not having a comparison group of term pregnancies (or placentas) or pregnancies

uncomplicated by abruption or bleeding.(8, 44, 61, 115) This is important because both bleeding and infection are causes of preterm delivery, thus in this instance preterm delivery may be a collider variable – that is, a common effect of the two variables of interest.(118) Investigating the association between bleeding and infection only among preterm deliveries is a form of collider stratification, which can induce spurious associations between variables.

1.5. Aims

The aims of this dissertation are:

1. To evaluate four indicators of placental hemorrhage (first trimester bleeding, placental abruption, disc-impacting blood clots, and high scores on a Microscopic Vascular – Disturbance of Integrity construct) as potential components of a common "bleeding pathway" by (1) assessing their mutual associations, (2) describing their prevalence according to maternal characteristics, and (3) estimating their odds of preterm delivery and its subtypes;

2. To assess the associations between four polymorphisms in three candidate maternal genes in thrombophilia and renin-angiotensin system pathways and preterm deliveries subtypes defined by evidence of placental hemorrhage;

3. To compare histologic chorioamnionitis with clinical chorioamnionitis, and to evaluate risk of histologic chorioamnionitis and clinical chorioamnionitis in relation to evidence of placental hemorrhage ascertained at different times in pregnancy.

CHAPTER 2.

EVIDENCE OF PLACENTAL HEMORRHAGE AND PRETERM DELIVERY

2.1. Introduction

Several etiologic pathways have been implicated in preterm delivery (PTD), including infection, stress, abnormal uterine distension, and uterine bleeding.(3) Various types of direct or indirect evidence of placental hemorrhage (PH) may be manifestations of a uterine bleeding pathway. Vaginal bleeding in early to mid-pregnancy has been associated with PTD in many studies.(20, 21, 62) Placental abruption, or premature separation of a normally placed placenta, is a potentially severe pregnancy complication that may originate with bleeding into the decidua from a ruptured arteriole(119) and can result in major obstetric hemorrhage. Placental abruption is present in a larger proportion of preterm than term deliveries.(24, 29) Subchorionic or retroplacental bleeding detected by prenatal ultrasonography(63, 120) and hemosiderin (a breakdown product of old blood) in placental tissues detected through histologic examination(61) have also been associated with PTD, although these have not been reported on extensively.

It is unclear whether these variable manifestations of bleeding belong to a single pathway with a common set of maternal risk factors. Vaginal bleeding early in pregnancy has been linked to placental abruption(20, 31, 62), suggesting that bleeding and abruption -- both clinically evident indicators of PH -- may share common pathways. Many other maternal characteristics and clinical circumstances are shared risk factors for both PTD and placental abruption, including hypertension(121-123), elevated maternal serum alpha-fetoprotein (MSAFP)(52, 66, 68), African-American identity(124, 125),

smoking(122, 126), cocaine abuse(127), and low body mass index (BMI)(128, 129). Less has been published on maternal characteristics associated with other evidence of PH, such as early pregnancy bleeding(130) or placental pathology findings.

While placental abruption is rare, complicating approximately 1% of pregnancies(28), placental pathology findings consistent with abruption are more prevalent(131). We hypothesized that placental abruption may only represent a portion of the uterine bleeding that affects delivery timing – the "tip of the iceberg" – because subclinical gross or microscopic evidence of placental hemorrhage apparent only in placental examinations might also be related to PTD risk. The prospective Pregnancy Outcomes and Community Health (POUCH) Study collected multiple sources of evidence of PH, including mid-pregnancy maternal reports of prior vaginal bleeding episodes, clinical data from medical records and gross and microscopic placental pathology findings. The aim of this study was to evaluate four indicators of PH as potential components of a common "bleeding pathway" by (1) assessing their mutual associations, (2) describing their prevalence according to maternal characteristics, and (3) estimating their odds of PTD and its subtypes.

2.2. Methods

Study Protocol

The POUCH Study enrolled 3019 pregnant women from five Michigan communities at 15-27 weeks' gestation. English-speaking women aged ≥15 years who had MSAFP screening at 15-22 weeks, a singleton pregnancy with no known congenital anomalies, and no pre-existing diabetes were eligible. MSAFP was of particular interest

in the POUCH Study's design because of this biomarker's consistent association with PTD(6); thus, the POUCH cohort oversampled women who had unexplained high MSAFP (≥2 multiples of the median, 7% of cohort). The POUCH study protocol received institutional review board approval from Michigan State University, the Michigan Department of Community Health, and all nine delivery hospitals. All women provided informed written consent.

At enrollment, women participated in a structured interview with a study nurse and completed a self-administered questionnaire. The interview elicited information on demographics (including self-reported race/ethnicity), height, pre-pregnancy weight, reproductive history, medical conditions and events during pregnancy. During the interview, women were asked, "Have you had any spotting or bleeding so far during this pregnancy?" and prompted to describe timing (gestational week) and heaviness (spotting, slight, about the same as usual period, or heavier than usual period) for up to 7 episodes. The questionnaire was designed to collect data on potentially sensitive questions including substance use and physical abuse. Women were asked how often in the last 6 months they had been "shoved, hit, or physically abused by [their] parents or a partner or husband" (never, once or twice, several times, often, or very often).

Gestational age was estimated by last menstrual period (LMP), corroborated by an ultrasound (US) scan conducted prior to 25 weeks (available for 97% of women). If the estimates differed by more than 2 weeks (17%) or if LMP was unavailable (3.6%), the ultrasound date was used. PTD was defined as delivery prior to 37 completed weeks' gestation.

Subcohort Sample

A subcohort (n=1371) was selected for detailed study of biologic samples and chart-level data. The subcohort included all PTDs, all women with unexplained high MSAFP and a stratified sample of women with normal MSAFP and term deliveries, with oversampling of African-Americans. Subcohort analyses incorporate sampling weights to reconstitute the cohort distributions and further correct for overrepresentation of high MSAFP in the cohort, such that weighted proportions based on the subcohort can be interpreted as prevalence or risk.

Placenta protocol

After subcohort deliveries, placentas were formalin fixed at the delivery hospital prior to transport to the study's pathology laboratory. For the gross examination, parallel slices were made through the placental disc 1 cm apart. The pathologist noted clots in the cut surface, along with indicators of adjacent tissue involvement, i.e. dissecting hemorrhage, and tissue infarction, compression, or red/brown discoloration. *Disc-impacting blood clot* was defined as a gross examination finding of a retro- or intraplacental blood clot impacting adjacent tissue.

For the microscopic placental examination, nine tissue samples per placenta were selected: two from the membrane roll, two from the cord, and five from the disc(19). Microscopic vascular-related findings that fell within five constructs adapted from a diagnostic coding tool were recorded. Items in the "Maternal Vascular – Disturbance of Integrity" (MV-I) construct, i.e. 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, were used to

calculate an MV-I score for each woman. *Microscopic hemorrhage* was defined as the top quintile of MV-I scores based on the distribution of scores among term deliveries with normal MSAFP. This serves as a possible indicator of atypical maternal vessel hemorrhage. This distributional cut-point along a continuum of findings was previously shown to correlate with PTD risk(23).

Medical record abstraction

Study nurses abstracted subcohort medical records in detail. For all PTDs, an additional brief abstraction was completed by a physician with expertise in obstetrics. A pool of possible placental abruption cases, identified by bleeding near delivery or any mention of placental abruption in the abstracted data, were later reviewed by three clinicians with labor and delivery experience who were unaware of the placental pathology findings recorded by the POUCH Study pathologist (PKS). Placental abruption was defined as (1) documented signs and symptoms consistent with abruption (e.g. vaginal bleeding, pain, increased uterine tone, fetal distress); or (2) retroplacental hematoma visualized on a prenatal ultrasound scan. Disagreements among the reviewers were resolved by discussion with the principal investigator (CBH) and study pathologist (PKS) until a consensus was reached.

Other relevant information abstracted from patient charts included trauma or injuries during pregnancy, episodes of vaginal bleeding during pregnancy and date of occurrence, blood pressure and proteinuria values, and details of the delivery process including timing of membrane rupture, cervical dilatation, and interventions. Hypertension was defined as documented diastolic blood pressure ≥90 and/or systolic blood pressure ≥140 on ≥2 days or a documented diagnosis and/or history of

hypertension, or anti-hypertensive medication prior to 20 weeks. Preeclampsia, gestational hypertension and chronic hypertension were not considered separately because numbers of each were small. PTD subtypes, including medically indicated, spontaneous labor, and premature rupture of membranes (PPROM) were determined based on chart-level data as previously described(14). Spontaneous PTD included spontaneous preterm labor and PPROM.

Evidence of placental hemorrhage

The four principal PH indicators compared in this study were (1) placental abruption, (2) disc-impacting blood clots, (3) microscopic hemorrhage, and (4) first trimester bleeding (any vs. none, ascertained during enrollment interview). Two additional variables describing vaginal bleeding during pregnancy were used instead of first trimester bleeding (any vs. none) in selected analyses: (1) first-trimester bleeding from the enrollment interview categorized as heavier than spotting, spotting only or none, and (2) first and second trimester vaginal bleeding (excluding bleeding in the week before delivery) recorded in prenatal charts categorized as first trimester only, second trimester only, both trimesters, or none.

Study Sample

From the subcohort, we sequentially excluded 158 women whose placentas were not saved, 127 women whose placental exams were not yet complete, 6 women with placenta previa at delivery, and 84 women who identified themselves as any race/ethnic category other than "White or Caucasian" (referred to as "white") or "Black or African-American" (referred to as "African-American"). After these exclusions, a sample of 996 women remained.

Descriptive statistics

As a first step, we calculated the prevalence of the four PH indicators and maternal characteristics overall and stratified by delivery timing (term/preterm). Next, we calculated the prevalence of each PH indicator among women with each of the other three indicators, and calculated bivariate odds ratios (OR) and 95% confidence intervals (CI) for each pair of PH indicators. Finally, we estimated the prevalence of each indicator according to selected maternal characteristics, and tested for differences in proportions using modified Rao-Scott χ^2 tests for complex survey designs(132).

Modeling strategy

We used weighted logistic regression models to assess relations between the four PH indicators and PTD. We calculated ORs for each PH indicator individually, then entered all four variables into the same model (i.e., a "mutually adjusted" model). Next, we added maternal characteristics to the model, and retained those that changed ORs for any of the PH indicators by ≥5% from the mutually adjusted estimates. Potential confounders considered were those associated with PTD or any of the PH indicators in univariate analyses. Finally, we added hypertension separately to examine its effect on other variables in the model. Hypertension may have a direct, proximate effect on placental abruption, placental pathology findings, and delivery timing.

Weighted polytomous logistic regression models were used to assess the relations between PH indicators and PTD subtypes defined by timing (35-36 weeks, <35 weeks, term [referent]) or clinical circumstances (spontaneous, medically indicated, term [referent]; and spontaneous labor, PROM, medically indicated, term [referent]).

All statistical analyses were conducted using SAS 9.1.3 (Statistical Analysis Software, Cary, NC).

2.3. Results

Descriptive statistics

Characteristics of the subcohort sample are detailed in Table 2.1, overall and stratified by delivery timing. In weighted analyses, 10.7% of pregnancies ended in PTD. In the total sample, prevalence of the four PH indicators ranged from 2.0% for placental abruption and 5.6% for disc-impacting blood clots to about 20% for microscopic hemorrhage and first trimester bleeding. All four PH indicators were more prevalent among PTDs than term deliveries. Medicaid insurance, lack of high school education, spending > 1 hour per week in a smoky room, hypertension and parity/prior PTD were also associated with PTD in these univariate analyses.

Table 2.2 shows pairwise associations among the four PH indicators. Although placental abruption was associated with all three other PH indicators (versus disc-impacting blood clot, OR=5.5 (1.7, 17.3); versus microscopic hemorrhage, OR=2.3 (1.0, 5.5); versus first trimester bleeding, OR=3.4 (1.4, 8.5), less than 50% of placental abruption cases had each of these other PH indicators (23.0%, 37.0%, and 43.8%, respectively). Disc-impacting blood clot and microscopic hemorrhage were strongly associated with one another (OR=4.6 (2.3, 8.9)), but first trimester bleeding was not associated with disc-impacting blood clot or microscopic hemorrhage (ORs 1.1 and 0.9).

Figure 2.1 shows the number of women with all combinations of the four PH indicators. A total of 413 women (38.9% weighted) had at least one PH indicator, and

321 women (31.9% weighted) had only one PH indicator. Nine of 31 placental abruption cases (35.2% weighted) had no other PH indicators.

Maternal characteristics had few statistically significant associations with evidence of PH (Table 2.3). Women with <12 years of education had a lower prevalence of first trimester bleeding than women with more education. Women with high MSAFP had a higher prevalence of both placental abruption and first trimester bleeding than women with normal MSAFP. Smokers had a lower prevalence of microscopic hemorrhage than non-smokers. Hypertensive women had a higher prevalence of disc-impacting blood clots than non-hypertensive women. Nulliparous women had a lower prevalence of all four PH indicators than parous women with and without prior PTD, although only the association with placental abruption was statistically significant.

Model Results

For PTD <37 weeks, there were elevated odds for each PH indicator in unadjusted analyses (Table 2.4). In a mutually adjusted model (i.e., a model that included all four PH indicators), all four ORs were attenuated, and disc-impacting blood clot lost statistical significance. Only minor changes occurred after adding maternal characteristics to the model. Adding hypertension to the model slightly attenuated the ORs for placental abruption and disc-impacting blood clot but had no impact on the other two PH indicators. When we considered two alternative specifications of vaginal bleeding during pregnancy in these models, we found that heavier bleeding had a stronger association with PTD than spotting, and bleeding in both first and second trimesters (from patient charts) had a stronger association with PTD than bleeding confined to either the first or second trimester in unadjusted but not adjusted analyses (not shown). However, neither

of these alternative specifications changed the interpretations for the other three PH indicators, thus all further analyses use first trimester bleeding (any vs. none) from the maternal interviews.

When we stratified PTD by gestational week, all four PH indicators had stronger associations with PTDs at <35 weeks than with PTDs at 35-36 weeks in all models (Table 2.4). All four PH indicators were significantly associated with PTD <35 weeks in all models.

When we stratified PTD by delivery circumstances, ORs did not differ meaningfully between spontaneous and medically indicated PTDs in unadjusted, mutually adjusted, or maternal characteristics-adjusted models (Table 2.5). After adding hypertension, the OR of 2.2 for disc-impacting blood clot and medically indicated PTD observed in the maternal characteristics-adjusted model was reduced to 1.1. Although numbers were very small in some cells, we further subdivided spontaneous PTDs into spontaneous labor and PPROM. Only microscopic hemorrhage was associated with PPROM (adjusted OR = 2.0, 95% CI 1.1, 3.5); the other three measures had stronger associations with spontaneous labor (not shown).

Table 2.1. Indicators of placental hemorrhage, maternal characteristics and risk factors, overall and by preterm delivery status

	Total		Term		Preterm		*P* †
	N	%*	N	%*	N	%*	
Total	996		758	89.3	238	10.7	
Indicators of Placental hemorrhage (not mutually exclusive)							
Placental abruption	31	2.0	12	1.4	17	7.3	<.0001
Cut surface clot impacting adjacent tissue	62	5.6	36	5.0	26	10.4	.005
Microscopic hemorrhage	215	20.4	143	19.3	72	29.4	.002
First Trimester Vaginal Bleeding	220	19.2	154	18.2	66	27.8	.005
Maternal characteristics							
African-American	416	24.6	340	23.6	76	32.9	‡
Medicaid-insured	536	45.6	411	44.8	125	52.7	.03
Education: did not complete high school	203	16.3	153	15.7	50	21.6	.03
High MSAFP	159	3.5	128	3.1	31	7.1	‡
Smoked during pregnancy	282	26.8	209	26.3	73	30.6	.23
Smoked prior to pregnancy	343	33.1	250	32.4	93	38.5	.10
Smoky room > 1 hr/wk	479	44.9	349	43.6	130	55.6	.002
Cocaine use (ever)	82	8.5	25	10.7	57	8.3	.29
Trauma/injury noted in medical record	116	10.7	89	10.6	27	11.8	.62
Physical abuse (previous 6 months)	105	9.1	83	9.1	22	8.8	.89
Hypertensive disorder (includes preeclampsia, pregnancy-induced hypertension, and chronic hypertension)	99	8.6	61	7.7	38	16.3	<.01
Age							
<20	160	13.3	122	12.9	38	16.3	0.36
20-34	765	78.6	583	78.8	182	77.0	
35+	71	8.1	53	8.3	18	6.7	
Parity/prior preterm birth							
Nulliparous	407	40.8	304	40.5	103	43.6	<0.0001
Parous/no prior preterm birth	449	49.2	380	51.7	69	28.6	
Prior preterm birth	139	9.9	74	7.8	65	27.7	
Prepregnancy Body Mass Index							
<18.5	44	3.6	30	3.3	14	6.1	0.13
18.5 - 24.9	438	45.4	336	45.8	102	42.5	
25-29.9	228	24.7	179	25.1	49	21.3	
≥30	286	26.3	213	25.8	73	30.1	

*Percentages have been weighted (inverse of sampling probability) to reflect distribution in cohort.
†P-value for preterm vs. term comparison by Rao-Scott chi-square test (SAS PROC SURVEYFREQ).
‡P-value can not be calculated in univariate analyses because exposure and outcome are sampling strata.

Table 2.2. Prevalence of four indicators of placental hemorrhage among women with each of the other three indicators (weighted row percent), and odds ratios for bivariate comparisons

	Placental abruption		Disc-impacting blood clots		Microscopic hemorrhage		First trimester bleeding	
	%	OR (95% CI)	%	OR (95% CI)	%	OR (95% CI)	%	OR (95% CI)
Among all women	2.0		5.6		20.4		19.2	
Among women with placental abruption (n=31)	--		23.0	5.5 (1.7, 17.3)	37.0	2.3 (1.0, 5.5)	43.8	3.4 (1.4, 8.5)
Among women with disc-impacting blood clots (n=62)	8.4	5.5 (1.7, 17.3)	--		51.1	4.6 (2.3, 8.9)	20.7	1.1 (0.5, 2.4)
Among women with microscopic hemorrhage (n=215)	3.7	2.3 (1.0, 5.5)	13.9	4.6 (2.3, 8.9)	--		18.5	0.9 (0.6, 1.5)
Among women with first trimester bleeding (n=220)	4.6	3.4 (1.4, 8.5)	6.0	1.1 (0.5, 2.4)	19.6	0.9 (0.6, 1.5)	--	

Table 2.3. Prevalence (weighted row percent) of four indicators placental hemorrhage according to selected maternal characteristics

	Placental abruption	Disc-impacting blood clot	Microscopic hemorrhage	First trimester bleeding
Overall	2.0	5.6	20.4	19.2
Race/ethnicity				
African-American	3.2	5.9	18.9	18.2
White	1.7	5.4	20.9	19.5
Medicaid-insured				
Yes	2.2	5.7	17.7	16.9
No	1.9	5.4	22.7	21.1
Education				
<12 years	2.9	6.0	16.6	13.4*
≥12 years	1.9	5.5	21.1	20.3
MSAFP				
Unexplained high	5.7*	7.8	23.5	32.9*
Not high	1.9	5.5	20.3	18.7
Smoked during pregnancy				
Yes	1.1	4.6	15.0*	15.8
No	2.4	5.9	22.4	20.5
Smoked 6 months before pregnancy				
Yes	1.3	4.5	16.7	15.5
No	2.4	6.1	22.3	21.0
Time spent in smoky room				
≥1 hr/wk	2.3	5.5	19.2	19.2
<1 hr/wk	1.8	5.6	21.4	19.2
Ever used cocaine				
Yes	2.0	4.7	13.4	16.1
No	2.0	5.6	21.1	19.5

Table 2.3 (cont'd)

	Placental abruption	Disc-impacting blood clot	Microscopic hemorrhage	First trimester bleeding
Trauma/injury noted in medial record				
Yes	5.4	3.6	17.5	17.0
No	1.6	5.8	20.8	19.5
Physical abuse (previous 6 months)				
Yes	1.5	8.1	23.0	21.7
No	2.1	5.3	20.1	18.9
Hypertensive disease				
Yes	4.0	15.3*	24.3	19.3
No	1.9	4.6	20.0	1.92
Age				
<20	2.1	5.4	20.7	14.5
20-34	2.1	5.4	20.3	19.8
35+	1.4	7.0	20.7	21.1
Parity/prior preterm birth				
Nulliparous	0.8*	4.4	18.5	17.6
Parous/no prior preterm birth	2.7	6.1	20.6	19.3
Prior preterm birth	3.9	7.3	27.3	25.6
Prepregnancy Body Mass Index				
<18.5	7.1	7.7	16.3	20.1
18.5 - 24.9	2.1	4.5	21.7	21.3
25-29.9	1.7	6.6	20.7	20.6
≥30	1.5	6.0	18.5	14.1

*P<.05 from Rao-Scott modified chi-square test.

Table 2.4. Odds ratios (OR) and 95% confidence intervals (CI) for the association between four indicators of placental hemorrhage and preterm delivery (PTD), overall and stratified by delivery timing (N=996).

	PTD (n)	Row total (n)	Unadjusted		Mutually Adjusted*		Adjusted for maternal characteristics†		Adjusted for maternal characteristics and hypertension‡	
			OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Preterm Delivery <37 weeks §	**238**									
Placental abruption	19	31	**5.2**	**2.4, 12.8**	**4.2**	**1.8, 9.8**	**4.0**	**1.7, 9.7**	**3.8**	**1.5, 9.5**
Disc-impacting blood clot	26	62	**2.2**	**1.3, 4.0**	1.7	0.9, 3.1	1.8	1.0, 3.2	1.5	0.8, 2.9
Microscopic Hemorrhage (top quintile)	72	215	**1.7**	**1.2, 2.5**	**1.6**	**1.1, 2.3**	**1.6**	**1.1, 2.4**	**1.6**	**1.1, 2.3**
First Trimester Bleeding	66	220	**1.7**	**1.2, 2.5**	**1.6**	**1.1, 2.4**	**1.7**	**1.2, 2.5**	**1.7**	**1.2, 2.5**
Preterm Delivery Stratified by Timing										
<35 Weeks ‖	**77**									
Placental abruption	11	31	**10.2**	**3.9, 26.5**	**6.4**	**2.4, 17.4**	**5.5**	**1.7, 17.2**	**5.3**	**1.7, 17**
Disc-impacting blood clot	14	62	**4.2**	**2.0, 8.5**	**2.6**	**1.2, 5.5**	**2.8**	**1.3, 6.0**	**2.4**	**1.1, 5.3**
Microscopic Hemorrhage (top quintile)	33	215	**3.0**	**1.8, 4.9**	**2.4**	**1.4, 4.1**	**2.5**	**1.4, 4.3**	**2.5**	**1.4, 4.3**
First Trimester Bleeding	25	220	**2.1**	**1.2, 3.5**	1.8	1.0, 3.2	**1.9**	**1.1, 3.5**	**1.9**	**1.0, 3.5**
35-36 Weeks ‖	**161**									
Placental abruption	8	31	**3.5**	**1.3, 9.6**	**3.0**	**1.1, 8.4**	**3.1**	**1.1, 8.8**	2.9	1.0, 8.5
Disc-impacting blood clot	12	62	1.4	0.7, 2.9	1.2	0.6, 2.7	1.3	0.6, 2.7	1.1	0.5, 2.5
Microscopic Hemorrhage (top quintile)	39	215	1.3	0.8, 2.0	1.2	0.8, 1.9	1.3	0.8, 2.0	1.3	0.8, 2.0
First Trimester Bleeding	41	220	1.6	1.0, 2.4	1.5	1.0, 2.4	**1.6**	**1.1, 2.5**	**1.6**	**1.1, 2.5**

*Model includes all four types of evidence of placental hemorrhage

†Model includes all four types of evidence of placental hemorrhage, maternal race, marital status, and BMI (categorical)

‡ Model includes all four types of evidence of placental hemorrhage, maternal race, marital status, BMI (categorical), and hypertension.

§OR and 95% CI calculated using weighted logistic regression models.

‖ OR and 95% CI for PTD in both time intervals calculated simultaneously using weighted logistic regression models.

NOTE: Boldface denotes P<0.05.

Table 2.5. Odds ratios (OR) and 95% confidence intervals (CI) for the association between four indicators of placental hemorrhage and spontaneous or medically indicated preterm delivery (PTD)

	PTD (n)	Row total (n)	Unadjusted		Mutually Adjusted*		Adjusted for maternal characteristics†		Adjusted for maternal characteristics and hypertension‡	
			OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Preterm Delivery, spontaneous §	**169**									
Placental abruption	13	31	**5.4**	**2.2, 13.2**	**4.3**	**1.7, 10.6**	**4.0**	**1.6, 10.1**	**3.9**	**1.5, 10.0**
Disc-impacting Blood Clot	17	62	2.0	1.0, 3.8	1.5	0.8, 3.0	1.6	0.8, 3.1	1.7	0.9, 3.4
Microscopic Hemorrhage, top quintile	51	215	**1.7**	**1.1, 2.5**	**1.6**	**1.0, 2.4**	**1.6**	**1.1, 2.5**	**1.6**	**1.1, 2.5**
First Trimester Bleeding	44	220	**1.6**	**1.1, 2.4**	1.5	1.0, 2.3	**1.6**	**1.0, 2.5**	**1.6**	**1.1, 2.5**
Preterm Delivery, indicated §	**69**									
Placental abruption	6	31	**5.9**	**2.0, 18.1**	**4.1**	**1.3, 13.1**	**4.0**	**1.2, 13.9**	3.0	0.6, 14.6
Disc-impacting Blood Clot	9	62	**2.9**	**1.3, 6.6**	2.2	0.9, 5.2	2.2	0.9, 5.3	1.1	0.4, 3.4
Microscopic Hemorrhage, top quintile	21	215	1.8	1.0, 3.2	1.6	0.9, 2.8	1.6	0.9, 3.0	1.7	0.8, 3.2
First Trimester Bleeding	22	220	**2.0**	**1.2, 3.6**	**1.9**	**1.1, 3.4**	**2.1**	**1.2, 3.9**	**2.2**	**1.1, 4.3**

*Model includes all four types of evidence of placental hemorrhage

†Model includes all four types of evidence of placental hemorrhage, maternal race, marital status, and BMI (categorical)

‡ Model includes all four types of evidence of placental hemorrhage, maternal race, marital status, BMI (categorical), and hypertension.

§OR and 95% CI for both PTD subtypes calculated simultaneously using weighted logistic regression models.

NOTE: Boldface denotes P<0.05.

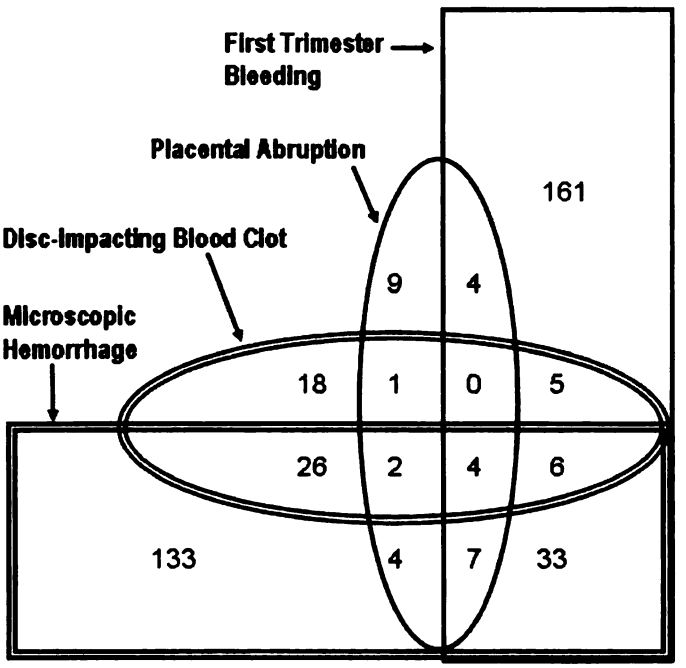

Figure 2.1. Venn diagram showing 15 possible combinations of the 4 indicators of placental hemorrhage. Disc-impacting blood clot = oval, double line, microscopic hemorrhage = rectangle, double line; first trimester bleeding = rectangle, single line; placental abruption = oval, single line. Note: 583 women with none of the 4 types of evidence are not represented.

2.4. Discussion

A key finding of this study was that evidence of PH identified through objective gross and microscopic placental pathology exams (i.e. disc-impacting blood clot and microscopic hemorrhage) were associated with PTD at <35 weeks even after accounting for clinically evident bleeding (i.e. placental abruption and first trimester bleeding). Previously, Salafia et. al. found that hemosiderin in the decidua or extraplacental membranes was more common in very preterm (<32 weeks' gestation) deliveries than in a sample of uncomplicated, healthy term deliveries in an unadjusted analysis(61). Associations between early pregnancy vaginal bleeding and subsequent PTD(20, 21, 62, 133) or placental abruption(29, 31, 62) have previously been identified. Our study builds on these prior findings by considering these clinically evident manifestations of placental hemorrhage in conjunction with subclinical placental pathology findings.

Prior studies have shown that pathology findings consistent with placental abruption are more common than clinical diagnoses.(119, 134) Some have argued that placental pathology-based abruption-related findings are inconsequential in the absence of clinical suspicion, given that most pregnancies with such findings have unremarkable outcomes(28). In the POUCH Study, two types of evidence of placental hemorrhage from pathology exams – disc-impacting blood clot (prevalence 5.6%) and microscopic hemorrhage ("prevalence" 20.4%, based on a distributional cut-point) – were clearly more common than placental abruption cases (prevalence 2.0%), and the majority of women with disc-impacting blood clots and high microscopic hemorrhage scores delivered at term without placental abruption. However, after accounting for placental abruption cases in multivariable models, we identified excess PTD risk associated with

both of these placental pathology findings, particularly for PTDs occurring at <35 weeks. Few studies have empirically demonstrated an association between subclinical evidence of hemorrhage in the delivered placenta and PTD by comparing preterm and term placentas. In prior work from the POUCH Study(23), the microscopic hemorrhage construct was found to be associated with both spontaneous and medically indicated PTDs occurring at <35 weeks. However, information on prior vaginal bleeding episodes and placental abruption were not considered in that study, thus it was unknown whether the observed results were primarily attributable to clinically evident hemorrhage.

Since retroplacental clots are sometimes employed in placental abruption clinical diagnoses, and have been required to confirm placental abruption cases in some epidemiologic studies(39, 45, 112), the limited association between placental abruption and disc-impacting blood clots is noteworthy. It is important to distinguish the disc-impacting blood clot construct captured by the POUCH Study pathologist from adherent retroplacental clots visualized on a freshly delivered placenta by an attending clinician. Data on the latter is not uniformly recorded in patient charts, thus we relied on a gross placental pathology protocol to infer presence of a clot prior to delivery. Collected blood not resulting from hemorrhage can become sequestered in the space between the membranes and disc and become firm with formalin fixation, resembling a true clot from hemorrhage. Thus, the pathologist identified clots associated with disc tissue changes that would support a significant retroplacental clot on gross examination alone. Disc-impacting blood clots undiagnosed as placental abruption may signal concealed (possibly intraplacental or dissecting) hemorrhage, a less severe clinical picture, or a case with a low index of suspicion for placental abruption. Placental abruption diagnoses in the

absence of disc-impacting blood clots could occur in very acute abruptions in which clots do not have time to organize and cause tissue reactions prior to delivery(135).

We found that microscopic hemorrhage was modestly associated with PTD (unadjusted OR=1.7), and was minimally affected by adjustment for the other types of evidence of placental hemorrhage, maternal demographic characteristics, or hypertension. While it is possible that findings in this construct represent intermediate steps on a pathway that sometimes leads to overt placental abruption, most cases of placental abruption (63%, see Table 2) did not have high scores on this construct. We suspected that gestational vaginal bleeding remote from delivery, which has been linked to placental abruption and PTD in this and other studies(20, 21, 29, 31, 62, 136), might signal chronic, slow hemorrhage, and would thus be associated with high microscopic hemorrhage scores, but this was not the case. Microscopic hemorrhage was associated with PPROM, while the three other manifestations of placental hemorrhage were not. Possible mechanisms linking specific items included in our microscopic hemorrhage construct to spontaneous PTD and PPROM have previously been suggested: thrombin generated in response to decidual hemorrhage may cause cervical ripening, contractions, and membrane degradation resulting in preterm labor or PPROM(103); and tissue hemosiderin, an iron compound produced during the breakdown of blood, may exert tissue irritant and pro-inflammatory effects that contribute to preterm labor(33). Additional research is needed to discover the antecedents of high microscopic hemorrhage scores.

The strengths and limitations of this study primarily derive from the prospective cohort design and the placental pathology protocol. Statistical power to detect significant

associations differed among the four PH indicators given their widely varying prevalence, which ranged from 2.0% to 20.4%. Sample size limitations precluded assessing risk of very early PTDs (i.e. <32 weeks). However, performing the pathology exam on a large sample of term and preterm deliveries allowed us to empirically evaluate the relations between PTD and four PH indicators. Like most studies that have investigated gestational vaginal bleeding in relation to pregnancy outcome, we cannot discern the actual origin of the blood – some may be from the placenta, while other reported bleeding may have originated in the cervix or vaginal tract. The study population was drawn from a well-characterized cohort, which was very similar to community women giving birth in the study years based on birth certificate comparisons.(137) Data on first trimester bleeding and substance use were ascertained prospectively, therefore this information was not subject to differential reporting based on mother's or clinician's knowledge of pregnancy outcome. The subcohort sampling scheme is not a source of bias because appropriate weighted analyses were performed.

Each of the four manifestations of PH considered in this study contributed information regarding PTD risk. Some of the PH indicators were associated with one another, but others were not. Many placental abruption cases did not show strong evidence of PH in their placental pathology examinations. The PH indicators diverged somewhat with respect to their relations with maternal characteristics and PTD subtypes. Taken together, these findings suggest potential heterogeneity in the hypothesized PH "iceberg". In the future, it may be helpful to consider both clinical and subclinical manifestations of PH in relation to biomarker data (e.g. gene polymorphisms, anti-

angiogenic factors) in order to gain insight into broader pathways to PTD that may involve disrupted uteroplacental vascular integrity.

CHAPTER 3.

POLYMORPHISMS IN THROMBOPHILIA AND RENIN-ANGIOTENSIN SYSTEM PATHWAYS, PRETERM DELIVERY AND EVIDENCE OF PLACENTAL HEMORRHAGE

3.1. 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.(3) Several pathways have been implicated in the etiology of PTD, including infection, stress, uterine distension, and uterine bleeding.(3) Placental abruption (PA), a rare pregnancy complication characterized by premature placental detachment, is strongly associated with PTD.(138) Moreover, other less severe concealed or subclinical placental hemorrhage evident only in placental pathology examinations may mark excess PTD risk(33). 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(56, 139), 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. Polymorphisms in these three genes that have functional effects on the gene in which they reside have all previously been investigated in relation to PA (see review and meta-analysis(83)). 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 associations(84, 95, 97, 98, 100, 140). Other studies have attempted to link thrombophilias to vascular placental pathology findings among placentas from complicated deliveries,(58, 101, 102) 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.

3.2. 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 defined by evidence from chart review by consensus of three clinician reviewers with labor and delivery experience. 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(19), without pathologist knowledge of gross examination findings. Severe histologic chorioamnionitis (HCA) was determined as previously described(19). Microscopic vascular-related findings that fell within five constructs adapted from a diagnostic coding tool were recorded(23). 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.(141, 142) 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

The current analysis includes white and black women with complete data on polymorphisms and placental pathology, and no placenta previa. 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 HCA.

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.).

3.3. Results

Race-specific minor allele frequencies are shown in Table 3.1. The minor alleles for MTHFR677, MTHFR1298, 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 3.2. 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%, respectively). 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 3.3 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 3.4. 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 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 HCA 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 3.5). 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).

Table 3.1. 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 3.2. 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	%‡
	959	100.0	30	2.1	93	9.5	836	88.4
Preterm delivery <37 weeks								
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
≥30	277	26.4	5	1.5	25	9.4	247	89.0

Table 3.2 (cont'd)

	Subcohort Sample		Evidence of Placental Hemorrhage					
			Placental Abruption		Subclinical Evidence*		None	
	N	%†	N	%‡	N	%‡	N	%‡
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	81	81.5
None	862	91.3	26	1.9	78	9.0	758	89.1
Prenatal vitamin use								
Started prior to pregnancy	124	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 3.3. 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 (1691)				
	GG	140	386	1.0	
	GA or AA	12	22	1.7	0.8, 3.7
	AGT(-6)				
	GG	50	136	1.0	
	GA or AA	102	272	1.0	0.7, 1.6
Black	**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 (1691)				
	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 3.4. 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

			Preterm Delivery Subtypes					
		Term	With Evidence of Placental Hemorrhage*			Without Evidence of Placental Hemorrhage*		
Race	Genotype	n	n	OR†	95% CI	n	OR†	95% CI
White	**Total**	408	28			124		
	MTHFR (677)							
	CC	324	13	1.0		49	1.0	
	TC or TT	236	15	0.8	0.4, 1.7	75	1.1	0.7, 1.7
	MTHFR (1298)							
	AA	259	10	1.0		60	1.0	
	AC or CC	301	18	1.7	0.7, 4.0	64	0.9	0.6, 1.4
	FVL (1691)							
	GG	526	23	1.0		117	1.0	
	GA or AA	34	5	**4.8**	**1.6, 14.2**	7	1.2	0.5, 3.0
	AGT(-6)							
	GG	186	5	1.0		45	1.0	
	GA or AA	374	23	**3.8**	**1.3, 10.5**	79	0.9	0.6, 1.53
Black	**Total**	328	18			53		
	MTHFR (677)							
	CC	322	16	1.0		41	1.0	
	TC or TT	77	2	0.5	0.1, 2.52	12	1.3	0.6, 2.6
	MTHFR (1298)							
	AA	220	14	1.0		34	1.0	
	AC or CC	108	4	0.6	0.2, 1.9	19	1.1	0.6, 2.1
	FVL (1691)							
	GG	396	18			53		
	GA or AA	3	0			0		
	AGT(-6)							
	GG	10	0			2		
	GA or AA	389	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).

NOTE: Boldface denotes P<0.05.

Table 3.5. 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								
		Term	With Placental Abruption			With Subclinical Evidence of Placental Hemorrhage*			With No Evidence of Placental Hemorrhage		
Race	Genotype	n	n	OR*	95% CI	n	OR*	95% CI	n	OR*	95% CI
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	0.4, 3.4	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	0.7, 5.6	64	0.9	0.6, 1.4
	FVL (1691)										
	GG	386	8			15			117		
	GA or AA	22	2	**5.4**	**1.0, 28.2**	3	**4.4**	**1.1, 16.9**	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**	**1.5, 17.2**	79	0.9	0.6, 1.3

Table 3.5 (cont'd).

Race	Genotype	Term	Preterm Deliveries: With Placental Abruption			Preterm Deliveries: With Subclinical Evidence of Placental Hemorrhage*			Preterm Deliveries: With No Evidence of Placental Hemorrhage		
		n	n	OR*	95% CI	n	OR*	95% CI	n	OR*	95% CI
Black	**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	0.1, 4.1	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	0.2, 3.8	19	1.1	0.6, 2.1
	FVL (1691)										
	GG	325	8			10			53		
	GA or AA	3	0			0			0		
	AGT(-6)										
	GG	8	0			0			2		
	GA or AA	320	8			10			51		

*Evidence of placental hemorrhage from the pathology exams in the absence of 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.

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

3.4. Discussion

In this study, we investigated MTHFR(677), MTHFR(1298), 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.(83) 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.(77) 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.(107, 143)

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.(144) One study conducted in Mexico identified an association between AGT M235T and preterm premature rupture of membranes(95), and only two studies have reported on AGT polymorphisms and abruption risk, with conflicting results.(45, 94) This variant has also been associated with preeclampsia(145) and hypertension outside of pregnancy.(82) Because PA has been consistently linked to high blood pressure,(73-76) it is unknown whether the association between AGT genotypes and PA observed in one large study(45) was attributable to hypertension. We speculate that local effects of the renin-angiotensin system in the decidual spiral arteries(146) 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(37, 38, 43, 57, 59, 90, 97, 140), although a few studies have identified positive associations with PA or other poor pregnancy outcomes(36, 98, 100). 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 true folate deficiency was probably rare. Direct measures of folate and homocysteine status were not available in the POUCH Study; however, we did not find any meaningful differences in our results after excluding women who took prenatal vitamins prior to conception. 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.(58, 101, 102) 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 associations(147). 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 comparions 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 error(148).

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.

CHAPTER 4.

CLINICAL CHORIOAMNIONITIS, HISTOLOGIC CHORIOAMNIONITIS AND EVIDENCE OF PLACENTAL HEMORRHAGE

4.1. Introduction

Infection and bleeding have been identified as two pathways to preterm delivery (PTD)(3, 7), although these pathways may not be independent. Chorioamnionitis, or inflamed fetal membranes, typically results from infected amniotic fluid and may be on a causal pathway to PTD(149). Sometimes chorioamnionitis produces clinical symptoms (i.e. clinical chorioamnionitis, CCA), but chorioamnionitis is more often subclinical and can only be identified by histologic examination of delivered placental tissue (i.e. histologic chorioamnionitis, HCA).

Early pregnancy vaginal bleeding and placental abruption (a severe bleeding-related pregnancy complication) occur more frequently in pregnancies that culminate in PTD (20, 21, 133, 138, 150-152). Several studies have linked placental abruption to CCA(29), other clinical evidence of infection(24, 105, 111), or HCA(31, 40, 44, 112, 115), but other studies have not concurred(113, 114). Few studies have specifically linked early bleeding with subsequent diagnosis of HCA or CCA(108, 109). Little is known about the mechanisms that connect early bleeding to PTD, but some have suggested that early bleeding is infection-related(108-110, 117).

Some evidence suggests that HCA and CCA are not necessarily concordant(153, 154), and we have identified no studies that consider both HCA and CCA in relation to placental abruption in the same sample of pregnant women. Thus, it is unclear whether associations with bleeding-related variables are similar for HCA and CCA.

Elevated maternal serum alpha-fetoprotein in the absence of fetal anomalies ("unexplained" high MSAFP), measured in mid-pregnancy, is one of the most consistent biomarkers of elevated PTD risk(6, 70, 71, 155). Mid-pregnancy high MSAFP levels are also associated with early pregnancy bleeding(156) and placental abruption(31, 52, 70, 71), but to our knowledge no studies have demonstrated an association with HCA. A key goal of the prospective Pregnancy Outcomes and Community Health (POUCH) study has been to understand what PTD pathways are marked by high MSAFP(6). The POUCH study collected multiple types of evidence of placental hemorrhage ascertained through mid-pregnancy maternal interviews, medical chart review, and placental histopathology exams. The aims of this study were to compare HCA and CCA, and to evaluate risk of HCA and CCA in relation to high MSAFP screening and evidence of placental hemorrhage ascertained early and late in pregnancy.

4.2. Methods

Study Protocol

The Pregnancy Outcomes and Community Health (POUCH) Study enrolled 3019 pregnant women at 15 through 27 weeks' gestation from 52 clinics in five Michigan communities in 1998-2004. Women carrying singleton pregnancies with no identified congenital anomalies at the time of enrollment were eligible if they were at least 15 years old, had maternal serum alpha-fetoprotein (MSAFP) screening at 15 through 22 weeks, did not have diabetes outside of pregnancy, and were proficient in English. The cohort oversampled women with unexplained high MSAFP, such that these constitute 7% of the cohort (estimated population prevalence, 3.5%). The study protocol received ethics

approval from Michigan State University and the Michigan Department of Community Health, and all women provided written informed consent. At enrollment, women were interviewed by a trained study nurse and filled out a questionnaire. These instruments collected data on demographics, height and pre-pregnancy weight, reproductive history, vaginal bleeding during the current pregnancy, medication use and substance abuse. Women were asked to select their primary race or ethnic heritage from a list.

Subcohort Sample

A subcohort (N=1371) was selected for in-depth study, using a sampling scheme designed to maximize efficient use of resources. The subcohort included all PTDs, all women with unexplained high MSAFP (≥2 MoM), and a stratified sample of women with term deliveries and normal MSAFP, with oversampling of African-American women in this latter category. Weighted analyses using the SURVEY procedures in SAS (version 9.1.3, Statistical Analysis Software, Cary, NC) were performed in order to appropriately account for the sampling scheme, so that weighted frequencies can be interpreted in relation to the cohort distributions (as prevalence or risk), additionally correcting for the oversampling of high MSAFP into the cohort.

Chart Abstraction and Clinical Diagnoses

For the subcohort, prenatal and labor and delivery records were abstracted in detail by study nurses. Gestational age was estimated by last menstrual period (LMP) and by an ultrasound scan performed prior to 25 weeks' gestation when available (97% of subcohort). If the two estimates differed by more than 2 weeks, the ultrasound estimate was used, otherwise the LMP estimate was used (79% of subcohort). PTD was defined as delivery occurring prior to 37 completed weeks. Other information abstracted from

patient charts included episodes of vaginal bleeding and vaginal infections during pregnancy, laboratory data including blood counts, and indicators of infection during labor.

Placental abruption and CCA were determined based on signs and symptoms recorded in patient charts. Placental abruption cases required either (1) significant bleeding prior to delivery or intrapartum without other cause, abdominal or back pain, uterine tenderness, or increased uterine tone; or (2) retroplacental hematoma visualized on a prenatal ultrasound scan. CCA was defined as a documented fever >100F, accompanied by at least two of the following four signs and symptoms: white blood count >15,000, uterine tenderness, foul-smelling vaginal discharge or amniotic fluid, and maternal or fetal tachycardia.

Gross Placental Pathology

For subcohort women, placentas were formalin fixed after delivery. Gross examination was performed using standard methods. Parallel slices were made through the placental disc 1 cm apart. Retroplacental or intraplacental blood clots visible on the cut surface with evidence of adjacent tissue involvement, i.e. dissecting hemorrhage, tissue infarction, compression, or red/brown discoloration were defined as *disc-impacting blood clots*.

Microscopic Placental Pathology

A detailed microscopic placental examination was performed using nine tissue samples per placenta: two membrane rolls, two sections of umbilical cord, and five full-thickness sections from the disc(19), without pathologist knowledge of gross examination findings. HCA was classified as severe (polymorphonuclear leukocyte inflammatory

pattern in chorionic plate and/or extraplacental membrane chorion and amnion, plus karyorrhexis or necrotizing inflammation), mild (not meeting criteria for severe but having at least one high-powered field with greater than 10 polymorphonuclear leukocytes), or none. Microscopic vascular-related findings that fell within five constructs adapted from a diagnostic coding tool were recorded. For the current 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. Findings were summed across all relevant placental samples to derive a score for each woman. 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 hemorrhage. In this study, we considered the top quintile of MV-I scores to serve as a possible indicator of atypical maternal vessel hemorrhage. An association between high MV-I scores using this distributionally-defined cutpoint and PTD was previously demonstrated(23).

Thus, two early and three late indicators of placental hemorrhage were considered. The early indicators were (1) first trimester bleeding from maternal interviews at 15-27 weeks (none, spotting only, or heavier bleeding) and (2) first and second trimester bleeding documented in patient charts (none, first trimester only, second trimester only, or both first and second trimesters). The late indicators were (1) placental abruption; (2) disc-impacting blood clots identified in the gross pathology examination; and (3) top quintile of MV-I scores from the microscopic pathology examination.

Analytic Strategy

The current study includes subcohort women with completed placental pathology exams who identified themselves primarily as "White or Caucasian" (N=580) or "Black or African-American" (N=416). Univariate associations between categorical maternal characteristics and HCA or CCA were determined using weighted frequency distributions and Rao-Scott chi-square tests(132). Multivariable logistic regression models were developed using forward and backward selection to identify maternal characteristics that had significant independent associations with HCA or CCA.

We estimated the risk of HCA and CCA among women with high MSAFP and early and late evidence of placental hemorrhage. For the CCA analyses, we simplified information on bleeding in pregnancy to binary variables (any vs. none) in light of the extremely limited number of cases meeting the criteria for CCA. We used weighted logistic regression with HCA or CCA as the dependent variable to estimate odds ratios (OR) and 95% confidence intervals (CI) for high MSAFP and early and late evidence of placental hemorrhage. We adjusted for maternal characteristics that had been identified as having significant independent associations with HCA or CCA in the previous step.

Although the sample size was limited, we explored two potential sources of heterogeneity of effects for HCA: PTD and race. Interactions between high MSAFP, evidence of placental hemorrhage and PTD or race were formally tested using likelihood ratio tests (LRT).

4.3. Results

Descriptive Statistics

The distributions of PTD and maternal characteristics in the sample and the prevalence of HCA and CCA according to these characteristics are shown in Table 4.1. Overall, 238 (10.7%) women delivered preterm at <37 weeks, 77 (3.7%) delivered at <35 weeks, and 28 (1.4%) delivered at <32 weeks. Approximately one-quarter of the cohort was African-American, and 40.8% were nulliparous. Among subcohort women, 120 (weighted prevalence, 9.5%) had HCA and 17 (weighted prevalence, 1.4%) had CCA. Both HCA and CCA were strongly associated with PTD at <37, <35, and <32 weeks. Otherwise, HCA and CCA followed a different pattern of associations with maternal characteristics. Higher risk of HCA was observed for women who were African-American, had <12 years of education, were Medicaid-insured, were unmarried, or had a vaginal infection during the index pregnancy in univariate analyses. Because many of these characteristics are correlated, an adjusted model was developed (forward and backward selection produced similar results). Two variables were significantly associated with HCA in this model: race (African-American vs. white, OR=2.4, 95% CI=1.5, 4.0) and education (<12 years vs. ≥12 years, OR=1.8, 95% CI=1.0, 3.2) (not in table). For CCA, higher risk was observed for nulliparous women and smokers. When these two variables were entered in the same logistic regression model, both remained associated with CCA (nulliparous vs. parous, OR=8.6, 95% CI 2.6, 28.2; smoking, OR=4.2, 95% CI=1.2, 14.5, ORs not in table).

Table 4.2 shows the associations between CCA and HCA overall and stratified by PTD. Women with HCA had 2.6-fold greater odds of having CCA overall (95% CI 0.8,

7.9). This association was strong and statistically significant among PTDs (OR=7.2, 95% CI 1.9 to 27.1). HCA and CCA were not associated among term deliveries (OR=0.8, 95% CI=0.1, 6.7).

High MSAFP, Evidence of Placental Hemorrhage, and HCA

Table 4.3 shows the risk of HCA by high MSAFP and evidence of placental hemorrhage, with unadjusted and adjusted ORs. Unexplained high MSAFP was associated with HCA (aOR=1.7, 95% CI 1.0, 2.8). The absolute risk of HCA among women with high MSAFP was 15.6%. First trimester bleeding ascertained during mid-pregnancy maternal interviews was not associated with HCA. First trimester bleeding that continued into the second trimester as recorded in patient charts was associated with HCA (aOR=3.3, 95% CI 1.0, 11.6), although bleeding restricted to either the first or second trimester was not. The absolute risk of HCA among women with bleeding in both trimesters was 25.5%. None of the late evidence of placental hemorrhage was associated with HCA. We included both MSAFP and first and second trimester bleeding in the same model, and both remained significantly associated with HCA. Adjustment for race and education changed the ORs for these variables by <10% (not shown).

High MSAFP, Evidence of Placental Hemorrhage, and CCA

Table 4.4 shows the risk of CCA by high MSAFP and evidence of placental hemorrhage, with unadjusted and adjusted ORs. High MSAFP, early evidence of placental hemorrhage, and high MV-I scores were not associated with CCA. Two late indicators of placental hemorrhage – placental abruption and disc-impacting blood clots – were associated with increased risk of CCA. When we included placental abruption and disc-impacting blood clots in the same model, both remained significantly associated

with CCA. Adjustment for smoking and nulliparity changed the OR for disc-impacting blood clot by <10%, but it increased the OR for placental abruption by >10% (not shown).

Subgroup analyses

We tested whether and the associations with HCA were similar for preterm and term deliveries (not in table). The only significant interaction was between PTD and disc-impacting blood clot (LRT p <.001). In term deliveries, there was non-significantly lower risk of HCA for women with disc-impacting blood clots (3% vs. 8.9% in cohort term deliveries, OR=0.3, 95% CI=0.1, 1.3). In PTDs, there was higher risk of HCA among women with disc-impacting blood clots (29% vs. 14.5% in cohort PTDs, OR=2.8, 95% CI=1.1, 7.4.

We tested whether the associations with HCA were similar for white and black women (not in table). For high MSAFP and early evidence of placental hemorrhage, there were no significant interactions with race. There were significant interactions between race and late evidence of placental hemorrhage, i.e. placental abruption, disc-impacting blood clot, and high MV-I scores (LRT p<.01, p<.001, and p=.01, respectively). None of the 42 white women with HCA had placental abruption. Risk of HCA among white women with disc-impacting blood clot (1.2%) or high MV-I scores (3.2%) were somewhat lower than the background HCA risk in white women (6.9%). Risk of HCA among black women with placental abruption (26.9%) or disc-impacting blood clot (28.4%) were somewhat higher than the background HCA risk in black women (17.4%), but HCA risk among black women with high MV-I scores was not elevated (18.2%). In blacks, there were 1.8-fold greater odds of HCA among women with

placental abruption (95% CI 0.5, 6.1) and 2.0-fold greater odds of HCA among women with disc-impacting blood clot (95% CI 0.8, 4.8).

Table 4.1. Distribution of selected maternal characteristics in study sample and prevalence of histologic chorioamnionitis (HCA) and clinical chorioamnionitis (CCA) according to maternal characteristics.

	Subcohort Sample		HCA			CCA		
	n	%*	n	%†		n	%†	
Total	996	100.0	120	9.5		17	1.4	
Term Delivery	876	89.3	85	8.9		7	1.1	
Preterm Delivery								
<37 weeks	238	10.7	35	14.5	‡	10	4.1	‡
<35 weeks	77	3.7	18	23.8	‡	6	7.1	‡
<32 weeks	28	1.4	12	42.2	‡	6	19.6	‡
Race								
African-American	416	24.6	78	17.4	‡	6	1.3	
White	580	75.4	42	6.9		11	1.4	
Education								
<12 years	203	16.3	36	17.4	‡	5	2.3	
≥12 years	791	83.7	84	8.0		12	1.2	
Medicaid Insurance								
Yes	536	45.6	78	11.9	‡	9	1.3	
No	459	54.4	42	7.5		8	1.5	
Marital Status								
Unmarried	546	44.0	87	13.5	‡	7	1.1	
Married	449	52.6	33	6.4		10	1.6	
Parity								
Nulliparous	407	40.8	51	11.1		12	2.9	‡
Parous	589	59.2	69	8.4		5	0.4	
Smoked during pregnancy								
Yes	282	26.8	37	10.0		7	3.0	‡
No	714	73.2	83	9.3		10	0.8	

Table 4.1 (cont'd).

	Subcohort Sample		HCA			CCA	
	n	%*	n	%†		n	%†
Vaginal infection during pregnancy§							
Yes	312	24.6	53	13.8	‡	8	2.0
No	684	75.4	67	8.1		9	1.2
Age							
<20	160	13.3	22	12.8		2	1.7
20-35	765	78.6	89	9.0		12	1.1
≥35	71	8.1	9	8.7		3	3.7
Pre-pregnancy BMI							
Underweight	44	3.6	7	13.9		2	7.0
Normal Weight	438	45.4	57	10.2		9	1.6
Overweight	228	24.7	23	8.2		6	1.7
Obese	286	26.3	33	9.0		0	0

*Column percentages, weighted to reflect sampling scheme.

†Row percentages, representing incidence of HCA or CCA in cohort, weighted to reflect sampling scheme.

‡P-value<.05 from Rao-Scott chi-square test.

§Includes Trichomonas vaginalis, Neisseria Gonorrhea, Chlamydia trachomatis, or bacterial vaginosis

Table 4.2. Association between clinical chorioamnionitis (CCA) and histologic chorioamnionitis (HCA) overall and stratified by preterm delivery, N=996.

		HCA			
		Yes	**No**	**OR***	**95% CI**
All Deliveries					
CCA	**Yes**	6	11	2.6	0.8, 7.9
	No	114	865		
Preterm Deliveries (<37 Weeks)					
CCA	**Yes**	5	5	**7.2**	**1.7, 27.1**
	No	30	198		
Term Deliveries (≥37 Weeks)					
CCA	**Yes**	1	6	0.8	0.1, 6.7
	No	84	667		

*Analyses weighted to reflect sampling scheme.
NOTE: Boldface denotes P<0.05.

Table 4.3. Association between histologic chorioamnionitis (HCA) and unexplained high MSAFP and early and late evidence of placental hemorrhage.

	n/row total	HCA risk %*	Unadjusted		Adjusted†	
			OR*	95% CI	OR*	95% CI
Total	120/996	9.5				
MSAFP						
Unexplained high	23/159	15.6	**1.8**	**1.1, 2.9**	**1.7**	**1.0, 2.8**
EARLY EVIDENCE OF PLACENTAL HEMORRHAGE						
First trimester bleeding from midpregnancy maternal interviews						
None	94/776	9.9	1.0			
Spotting only	16/113	11.3	1.2	0.6, 2.5		
Heavier than spotting	10/107	8.4	0.9	0.4, 2.1		
First and second trimester bleeding from patient charts						
None	83/729	8.9	1.0		1.0	
First trimester only	21/159	11.5	1.3	0.7, 2.6	1.3	0.7, 2.5
Second trimester only	9/81	7.8	0.9	0.3, 2.2	0.9	0.3, 2.2
Both trimesters	7/27	25.5	**3.5**	**1.1, 11.2**	**3.4**	**1.1, 10.9**
LATE EVIDENCE OF PLACENTAL HEMORRHAGE						
Placental Abruption	4/31	10.4	1.1	0.3, 3.5		
Disc-impacting blood clot	9/62	8.3	0.9	0.4, 1.9		
Maternal Vascular - Disturbance of Intergrity score (top quintile)	22/215	6.6	0.6	0.4, 1.1		

*All analyses weighted to reflect sampling scheme.
†Model includes both indicators of placental hemorrhage that were significant in unadjusted model.
NOTE: Boldface denotes P<0.05.

Table 4.4. Association between clinical chorioamnionitis (CCA) and unexplained high MSAFP and early and late evidence of placental hemorrhage.

	n/row total	CCA risk %*	Unadjusted		Adjusted†	
			OR*	95% CI	OR*	95% CI
Total	17/996	1.4				
MSAFP						
Unexplained high	3/159	2.0	1.5	0.4, 5.6		
EARLY EVIDENCE OF PLACENTAL HEMORRHAGE						
First trimester bleeding from midpregnancy maternal interviews	5/220	1.3	0.9	0.3, 3.0		
First and second trimester bleeding from patient charts	6/267	1.9	1.6	0.4, 5.7		
LATE EVIDENCE OF PLACENTAL HEMORRHAGE						
Placental Abruption	4/31	7.2	**6.0**	**1.6, 22.8**	**4.4**	**1.3, 14.9**
Disc-impacting blood clot	9/62	4.9	**4.3**	**1.4, 13.4**	**3.6**	**1.2, 10.5**
Maternal Vascular - Disturbance of Intergrity score (top quintile)	22/215	0.7	0.5	0.1, 1.6		

*All analyses weighted to reflect sampling scheme.
†Model includes both indicators of placental hemorrhage that were significant in unadjusted model.
NOTE: Boldface denotes P<0.05.

4.4. Discussion

In this study, we found that HCA and CCA were not strongly concordant, followed different patterns of maternal characteristics, and were associated with different manifestations of placental hemorrhage. Two PTD risk markers observed in mid-pregnancy – unexplained high MSAFP and bleeding in both first and second trimesters – were associated with findings of severe HCA in the delivered placenta, but were not associated with CCA. CCA was associated with placental abruption and disc-impacting blood clots in the delivered placenta, but these late indicators of placental hemorrhage were not associated with HCA except in some subgroups of women, i.e. African-Americans and women who had preterm deliveries.

Many studies have reported that women with vaginal bleeding in early to mid-pregnancy have increased risk of PTD, through mechanisms not yet fully explained(20, 21, 62, 67, 133, 136, 150-152). MSAFP, an early biomarker associated with risk of PTD(70, 155), is also associated with early pregnancy bleeding(156). Our finding of increased HCA risk among women with bleeding in the first and second trimesters or high MSAFP suggests that these mid-pregnancy variables could actually mark an inflammation-related pathway. Few prior studies have linked bleeding and high MSAFP to measures of infection. A 2.6-fold increased risk of HCA but no increased risk of CCA were reported among 33 women with first or second trimester bleeding compared with 63 non-bleeding controls in a research letter.(109) A larger prospective cohort study reported that first trimester bleeding was associated with several vaginal infections and subsequent development of CCA(108), a finding we did not replicate. We only observed an association between bleeding and HCA when bleeding occurred in both the first and

second trimesters. A similar pattern has also been observed for vaginal bleeding in relation to PTD in some studies.(20, 21) We suspect that persistent bleeding and high MSAFP are both markers of ongoing placental problems that result from or support infection.

Our results showed no association between HCA and any late evidence of placental hemorrhage when all women were considered together. However, there was an association between HCA and disc-impacting blood clots among PTDs, and there were trends toward associations between HCA and both placental abruption and disc-impacting blood clots among black women. Histologic evidence of inflammation is objective and localizes inflammation to gestational tissues, but studies employing placental examinations have not generated consistent associations with placental abruption. Two cohort studies(40, 112) and two case-control studies(44, 115) identified positive associations, two case-control studies found no association(113, 114), and one cohort study had mixed (and overall very modest) results depending on the location of the inflammatory infiltrate(31). We found that associations between HCA and late indicators of placental hemorrhage differed by race, such that there was a trend toward increased risk of HCA with placental abruption and disc-impacting blood clots in black women but not white women. We find it plausible that populations with different underlying rates of HCA could have different relations between HCA and placental abruption. We also found that the association between HCA and disc-impacting blood clots differed by timing of delivery, such that an association between these two variables was only observed among PTDs. Differences in the results of studies to date may be attributable to location or severity of inflammation, the use of blinded research protocols or clinical

pathology exams for assigning HCA status, selection of controls for case-control studies (e.g. indicated preterm births, matching on parity), and study populations (e.g. high-risk or predominantly black population vs. general obstetric population). In addition, variability in placental abruption case definitions (e.g. studying only preterm placental abruption cases or requirement of a retroplacental clot) or differing criteria used to assign HCA status might also help to explain different results between studies.

Although only 17 women in the study sample met the criteria for CCA, we identified strong associations between this clinical measure of infection and both placental abruption and disc-impacting blood clot, a gross lesion consistent with premature placental detachment. We noted that HCA and CCA were strongly associated with one another among PTDs, but not associated at term. Previous case-control studies have reported imperfect correlation between these two manifestations of intrauterine infection(153, 154). Our results are concordant with several prior studies that have linked placental abruption with clinical evidence of infection, with measures of association ranging from 1.55 to 9.71(24, 29, 105, 111). We speculate that a spurious association between CCA and placental abruption could arise from the shared symptom of uterine tenderness. Other signs and symptoms of CCA are not specific to infections in the chorion and amnion (e.g. fever, leukocytosis) – these could indicate infection in other gestational or non-gestational tissues, or even a maternal response to placental abruption.

Many studies have considered infection a risk factor for placental abruption(24, 40, 44, 105, 112, 115), but time order is not clear. A few studies have suggested that bleeding during pregnancy might precede infection and (1) disrupt host defense mechanisms to enable microbes to ascend from the lower genital tract, (2) provide a

nutritive substrate for bacterial growth in previously colonized gestational tissues, or (3) allow microbes in the maternal circulation to gain access to gestational tissues and spaces(108, 110). In the POUCH study, although bleeding and high MSAFP were ascertained prior to determination of HCA status, it is not clear that disrupted uteroplacental vascular integrity preceeded infection. High MSAFP, for example, could be a marker of infection if infected membranes diffuse AFP more rapidly than healthy membranes.(6) High MSAFP levels could also result from increased transfer across the placenta attributable to a "breakdown in the placental fetal-maternal interface."(155) Bacteria may colonize the decidua and cause blood vessels to become friable, resulting in clinically evident bleeding or high MSAFP, thus these might be indicators of existing, otherwise clinically silent intrauterine infection.(108, 110, 117).

Generally, HCA results from bacteria ascending into the amniotic fluid, resulting in neutrophil migration toward the fetal membranes. The definition of severe HCA used in the POUCH Study incorporated information on number of neutrophils per high power field in the fetal membranes as well as grade of infection (i.e. presence of karyorrhexis or necrotizing inflammation). This definition previously identified women at markedly increased risk of PTD,(19) and it is quite severe compared to other studies investigating a link between HCA and placental abruption. It is possible that the inflammatory pattern most useful in discriminating women at elevated PTD risk differs from the inflammatory pattern that increases risk of (or results from) decidual hemorrhage or premature placental detachment. Presence of karyorrhexis and necrotizing inflammation may imply that the infection has been in place for some time (at least 24 hours). If placental abruption precedes HCA and the observed association in some studies is attributable to neutrophil

recruitment to the site of bleeding, inflammation may not have sufficient time to reach a high grade prior to delivery. The HCA definition used in this study did not incorporate information on decidual inflammation, as some studies have.(109, 112) As a next step, it may be helpful to consider acute and chronic decidual inflammation in relation to evidence of placental hemorrhage. It is possible that inflammation in the decidua might provoke a maternal response resulting in clinically evident infection more readily than inflammation confined to the fetal membranes. Studying inflammation in the decidua might help to reconcile our disparate results for HCA and CCA in relation to placental abruption, as well as to help understand reasons for the limited concordance between HCA and CCA.

Some limitations need to be considered when interpreting our findings. Given the cohort design and general obstetric population studied, we had a limited sample of placental abruption and CCA cases. We had 80% power to detect an OR of 3.3 between HCA and placental abruption. It is possible that, by chance, we obtained a sample in which no association between placental abruption and HCA existed (OR=1.1) when a true association exists. We calculated that in order to have 80% power to detect an OR of 2.5 (similar to another cohort study)(40), we would need to recruit an additional 918 women and observe 5-fold increased odds of HCA with placental abruption in this group. This is an extreme, but possible, scenario. We relied on data recorded in patient charts for several key variables, including second trimester bleeding, CCA, placental abruption, and vaginal infections in pregnancy. Given variability in prenatal practices, information may not have been documented equally for all women, and some degree of misclassification is possible.

Important strengths of this study include the availability for all women of an objective placental pathology examination, a screening MSAFP determination, and prospectively collected data on demographics, first trimester bleeding, and risk behaviors. The determination of HCA used in this study was based on a detailed examination by a pathologist who was masked to all clinical data and gross pathology findings. The pathologist also identified disc-impacting blood clots and microscopic vascular-related findings using a detailed descriptive protocol without any information on pregnancy outcomes. The ability to generalize our results is also a strength, helped by sampling that included women from several communities and practices, and with both high and low risk pregnancies.

This study provides limited support for the hypothesis that placental hemorrhage in pregnancy is related to infection or inflammation. Future studies should consider that associations between CCA or HCA and other variables may differ, because these are not the same entity. Reconciling differences between these manifestations of intrauterine infection and their disparate relations with bleeding-related variables may be helped by measuring deciduitis and its relations with CCA and HCA.

CHAPTER 5.

SUMMARY

The three studies presented in this dissertation have explored various facets of the epidemiology of placental hemorrhage. First, we determined that four manifestations of placental hemorrhage marked elevated preterm delivery risk, although they differed in their associations with some maternal characteristics and were not highly concordant with one another. Next, we found that women who were heterozygous for the Factor V Leiden variant or the AGT G-6A promoter polymorphism were at increased risk of a specific subset of preterm deliveries with evidence of placental hemorrhage, whereas they were not associated with preterm deliveries that had no evidence of placental hemorrhage. Finally, we found that early and late evidence of placental hemorrhage distinguished between clinical and histologic chorioamnionitis: bleeding in the first and second trimesters was associated with histologic chorioamnionitis, while placental abruption and disc-impacting blood clots were associated with clinical chorioamnionitis. We also identified some subgroup variations: associations between late indicators of placental hemorrhage and histologic chorioamnionitis differed for blacks and whites and differed between term and preterm deliveries.

Unexplained high MSAFP was featured in chapters 2 and 4. In chapter 2, we confirmed previous observations that high MSAFP is associated with first trimester bleeding and placental abruption. However, high MSAFP was not significantly associated with either of the placental pathology findings indicative of placental hemorrhage, although there were trends toward positive associations with both. In chapter 4, we found

that high MSAFP is associated with subsequent finding of HCA in the delivered placenta, even after accounting for early pregnancy bleeding. Few maternal systemic biomarkers have been identified for subsequent HCA. In a previous publication from the POUCH Study, associations between several circulating cytokines measured in mid-pregnancy maternal plasma and preterm delivery at <35 weeks with HCA, but not with HCA overall(157). The magnitude of the MSAFP-HCA association identified in this study is modest (OR=1.7), and the predictive ability is likely to be quite low. However, the association suggests that intrauterine infection may explain a portion of the consistent association between MSAFP and preterm delivery.

Placental abruption and disc-impacting blood clots are two sources of evidence of premature placental detachment. Findings from these three studies suggest that considerable heterogeneity may exist in the causes of this outcome. Some cases of placental abruption and disc-impacting blood clots may develop through thrombophilia or vascular function pathways, such as those marked by Factor V Leiden variant and the AGT(-6) GA/AA genotypes. Although we identified a very strong association between clinical chorioamnionitis and both placental abruption and disc-impacting blood clots, it is possible that the signs and symptoms of clinical chorioamnionitis actually occur in response to abruption and bleeding. When all women were considered together, there was no association between either measure of premature placental detachment and histologic chorioamnionitis, although our results suggested that an association may exist among black women or among women delivering preterm. Time order is not clear, and given the strong association between early bleeding (in the first and second trimesters) and subsequent HCA, it is possible that neutrophil infiltration occurs in response to bleeding.

However, it is also possible that in some subgroups of women, subclinical infection may precede and cause decidual bleeding which may lead to a retroplacental hematoma or overt placental abruption. A few studies have calculated an etiologic fraction for infection and abruption, and these have been quite low (Kramer: 2.6%(40), Ananth: 2.4% for preterm abruptions, 0.7% for term abruptions(24)). Thus, if infection is one cause of abruption, it may be a very uncommon cause.

Ultimately, the three studies presented in this dissertation all attempt to provide insight into pathways to preterm delivery that involve bleeding. Because associations with maternal characteristics, gene polymorphisms, and intrauterine infection differed between the measures of placental hemorrhage in many cases, we conclude that considerable heterogeneity exists even within the "bleeding pathway." Preterm deliveries may be marked by early or late bleeding for a number of reasons, and some of these reasons may overlap.

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