Associations between Maternal and Fetal Inherited Thrombophilias, Placental Characteristics Associated with Vascular Malperfusion, and Fetal Growth

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Abstract

Pregnancy results in alterations in coagulation processes, which may increase the risk of thrombosis. Inherited thrombophilia mutations may further increase this risk, possibly through alterations in the placenta, which may result in pregnancy complications such as poor fetal growth. The purpose of our study is to evaluate the association of fetal growth, approximated by birth weight for gestational age percentile, with genetic markers of thrombophilia and placental characteristics related to vascular malperfusion. We analyzed data from the Stillbirth Collaborative Research Network's population-based case–control study conducted in 2006–2008. Study recruitment occurred in five states: Rhode Island and counties in Massachusetts, Georgia, Texas, and Utah. The analysis was restricted to singleton, nonanomalous live births ≤ 42 weeks' gestation with a complete placental examination and successful testing for ≥ 1 thrombophilia marker (858 mothers, 902 infants). Data were weighted to account for oversampling, differential consent, and availability of placental examination. We evaluated five thrombophilia markers: factor V Leiden, factor II prothrombin, methylenetetrahydrofolate reductase A1298C and C677T, and plasminogen activator inhibitor type 1 in both maternal blood and placenta/cord blood. We modeled maternal and fetal thrombophilia markers separately using linear regression. Maternal factor V Leiden mutation was associated with a 13.16-point decrease in adjusted birth weight percentile (95% confidence interval: −25.50, −0.82). Adjustment for placental abnormalities related to vascular malperfusion did not affect the observed association. No other maternal or fetal thrombophilia markers were significantly associated with birth weight percentile. Maternal factor V Leiden may be associated with fetal growth independent of placental characteristics.

Keywords
► pregnancy
► inherited thrombophilias
► perinatal hemostasis

Introduction

Physiologic changes during pregnancy include alterations in coagulation processes in the preparation for potential bleeding at the time of delivery.[1] These physiologic changes also increase the risk of thrombosis.[1] Presence of an inherited thrombophilic mutation may further increase this risk and may be associated with pregnancy complications. Inherited thrombophilias include mutations that affect the coagulation cascade, which can increase the risk of venous thromboembolism (VTE).[2] Some of the more common mutations resulting in inherited thrombophilia include factor V Leiden (FV Leiden), factor II prothrombin (FII prothrombin), methylenetetrahydrofolate reductase (MTHFR), and plasminogen activator inhibitor type 1 (PAI-1).[2] Currently, the American College of Obstetricians and Gynecologists recommends screening for inherited thrombophilias only when a pregnant woman has a history of VTE or a first-degree relative with thrombophilia due to insufficient evidence linking inherited thrombophilias with pregnancy complications.[3]

Several studies have evaluated the relationship between inherited thrombophilias and fetal growth restriction, although results have been inconclusive. A systematic review published in 2005 reported that both FV Leiden and FII prothrombin are associated with an increased risk of fetal growth restriction; however, the authors caution that this association may be driven by low-quality studies with extreme associations.[4] A more recent systematic review of FV Leiden reported an association between maternal FV Leiden and small-for-gestational-age (SGA) infant.[5] Recent individual studies have reported conflicting results. A case–control study in Italy reported no association between FV Leiden, FII prothrombin, or MTHFR C677T and fetal growth restriction, determined from abnormal umbilical arterial blood flow.[6] Conversely, a similar, larger study also conducted in Italy reported that FV Leiden and FII prothrombin mutations in neonates, but not mothers, are associated with increased risk of fetal growth restriction in those without hypertension.[7] MTHFR mutations are associated with hyperhomocysteinemia,[8] which has been associated with fetal growth.[9] Conflicting results have been reported for PAI-1.[10–12] However, a recent case report suggests that this mutation is an independent risk factor for placental insufficiency.[13]

Potential associations between inherited thrombophilia and fetal growth restriction may be mediated by alterations in placental function, as the placenta is responsible for regulating fetal growth and development through mediating oxygen, nutrient, and waste transfer. The purpose of our study is to evaluate the association of fetal growth, approximated by birth weight for gestational age percentile, with genetic markers of thrombophilia and placental characteristics related to vascular malperfusion. We hypothesize that the relationship between placental characteristics related to vascular malperfusion and fetal growth may be driven in part by maternal and/or fetal thrombophilic mutations.

Materials and Methods

The Stillbirth Collaborative Research Network (SCRN) conducted a population-based case–control study of stillbirth. Participants delivering a stillborn or live born infant were enrolled between March 2006 and September 2008. Recruitment occurred at 59 hospitals representing five catchment areas of the United States: Rhode Island and counties in Massachusetts, Georgia, Texas, and Utah. Controls were sampled from all live births in these catchment areas using a stratified random method. The SCRN study enrolled 663 women with a stillbirth (cases) and 1,932 women with a live birth (controls). Details of the study design, including sampling methods, have been published elsewhere.[14] The study was approved by the Institutional Review Board at each study site.

Birth weight and gestational age were obtained from medical record abstraction. Birth weight percentile adjusted for gestational age was determined from Hadlock's growth curves.[15] We conducted a secondary analysis using logistic regression with SGA, defined as a birth weight less than 10th percentile for gestational age, as the outcome. Women had the option to consent to a placental examination, which was conducted by a perinatal pathologist using a standard protocol.[16] Of the 1,932 women with a live birth, 93.4% consented to a placental examination that was completed and considered adequate in 69.7%.[16] In 23.1% of those who consented to a placental examination, the placenta had been previously discarded and could not be examined.[16] The presence of placental characteristics related to maternal and fetal vascular malperfusion was determined from this examination. Characteristics potentially related to maternal vascular malperfusion include retroplacental hematoma, any parenchymal infarction (focal, multifocal, diffuse), intraparenchymal thrombus, and perivillous/intervillous fibrin/fibrinoid deposition. Characteristics related to fetal vascular malperfusion include fetal vascular thrombi in the chorionic plate, any avascular villi (focal, multifocal, diffuse), and edema (placental hydrops).

Samples were collected at the time of birth and stored at −80°C prior to assay. Testing for thrombophilia markers occurred 2 to 5 years after sample collection. Maternal thrombophilia markers were evaluated using maternal blood and fetal thrombophilia markers were evaluated using fetal blood from the umbilical cord and placental tissue. Samples were genotyped for four single nucleotide polymorphisms (FV Leiden, rs6025; prothrombin G20210A, rs1799963; MTHFR C677T, rs1801133; and MTHFR A1298C, rs1801131) and one deletion/insertion polymorphism (PAI 4G/5G, rs1799768) using TaqMan allelic discrimination chemistry (Life Technologies, Foster City, California, United States) with validated assays and the manufacturer's protocols. Specifically, 6 ng of DNA was included in a 5-μL reaction with a final concentration of ×1 TaqMan genotyping master mix and ×0.5 TaqMan genotyping assay mix. Details of DNA extraction and evaluation have been reported.[12] Genotypes were verified and the polymorphisms were evaluated for deviation from Hardy-Weinberg equilibrium. All samples were genotyped in duplicate to ensure

accurate genotyping. Laboratory personnel were blinded to the clinical status of the samples.[12]

We restricted our analysis to live births (controls) with a placental examination and successful thrombophilia testing for at least one thrombophilia maker in either maternal blood (mother) or placenta/cord blood (infant). We additionally excluded multiple gestations, anomalous pregnancies, and births more than 42 weeks' gestation. Our final sample included 858 mothers and 902 infants with successful testing for at least one marker (►Fig. 1).

Data weights were calculated to account for differential consent for enrolment, oversampling, and availability of the placental examination.[14] Weights for the availability of the placental examination incorporated clinic site, induction, time trend, delivery time, weekend delivery, and mode of delivery. We conducted weighted linear regression to evaluate the association of fetal growth, approximated by birth weight for gestational age percentile, with thrombophilia markers and placental characteristics. Analyses were conducted separately for maternal (the presence of thrombophilia markers in maternal blood with placental characteristics related to maternal vascular malperfusion) and infant (the presence of thrombophilia markers in placenta/cord blood with placental characteristics related to fetal vascular malperfusion) throm-

bophilia. Each of the five thrombophilia markers of interest was classified as present (individuals with one or two copies of the mutation) or absent (individuals with no copies of the mutation—common homozygous). We also created a summary variable to indicate number of thrombophilia markers present and analyzed this as a dichotomous variable (no markers present vs. one or more markers present). We controlled for maternal age and maternal race/ethnicity (self-reported) in the maternal and fetal models and additionally controlled for paternal age and paternal race/ethnicity in the fetal models, as these characteristics may confound the association between presence of thrombophilia markers and fetal growth. To evaluate differences in means and proportions, t-tests and chi-square tests were used, respectively. All statistical tests used a p-value of less than 0.05 to determine statistical significance. Analyses were performed using SUDAAN version 11.0 (Research Triangle Institute, Research Triangle Park, North Carolina, United States).[17]

Results

Descriptive Statistics
Our analytic population consisted of predominantly term births (91.6%; ►Table 1). The average birth weight for gestational age percentile was 42.99, with 71.0% of infants falling between the 10th and 90th percentiles. Roughly, three-quarters of mothers were between 20 and 34 years of age and 46.0% were non-Hispanic white. Similarly, 70.0% of fathers were between 20 and 34 years of age and 43.1% were non-Hispanic white. Over half of mothers had completed at least 1 year of college and 63.1% were married at the time of birth. Approximately half of mothers had a pre-pregnancy body mass index within the normal range (18.5–24.9) and 87.2% of mothers did not smoke in the 3 months prior to pregnancy.

Thrombophilia Markers and Fetal Growth
As expected, FV Leiden and FII prothrombin were relatively rare in this population (2.8 and 1.9% in mothers and 2.6 and 1.5% in infants, respectively; ►Table 2). The other markers were more common (PAI-1: 62.2% in mothers, 61.3% in infants; MTHFR A1298C: 38.5% in mothers and 40.3% in infants; MTHFR C677T: 55.5% in mothers and 54.2% in infants); 82.6% of mothers and 84.6% of infants had at least one marker. Maternal FV Leiden was associated with a lower mean birth weight percentile in crude analyses (absent: 43.20, present: 33.74), although this difference was not statistically significant. Conversely, those with fetal FV Leiden had a higher mean birth weight percentile (absent: 42.72, present: 49.20). Other thrombophilic polymorphisms were not associated with lower mean birth weight percentile, and the maternal PAI-1 genotype was associated with a statistically significantly higher mean birth weight percentile (absent: 38.85, present: 44.36).

Placental Characteristics and Fetal Growth
With the exception of parenchymal infarction, which occurred in 16.2% of pregnancies, and intraparenchymal thrombus, which occurred in 13.6% of pregnancies, placental changes related to thrombosis were rare (►Table 3). For
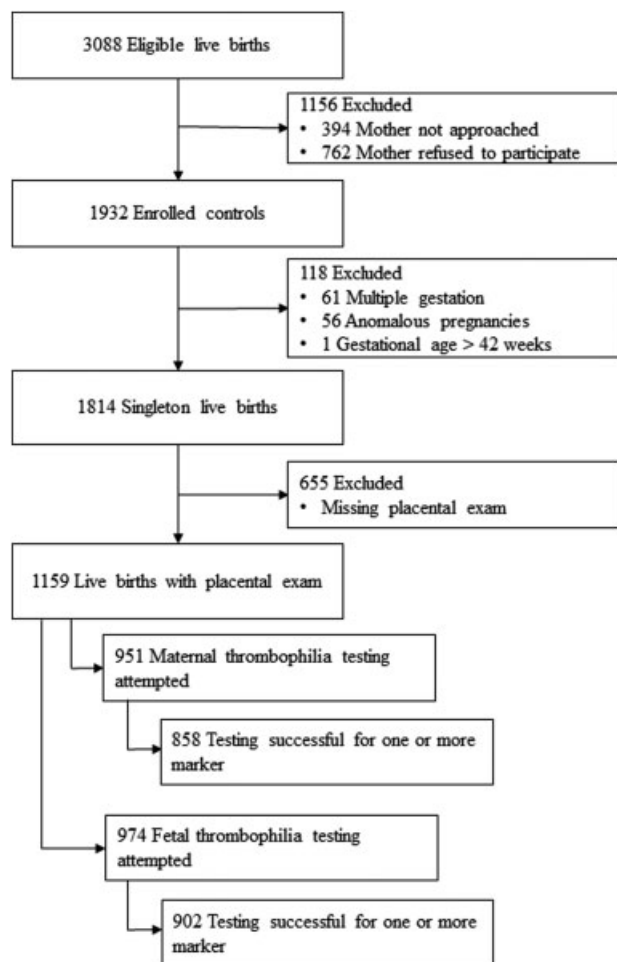

Fig. 1 Study enrollment and inclusion.

Table 1 Descriptive characteristics of study population

Characteristics	Mean (SE) or %$_w$
	$N = 1{,}073$
	$N_w = 882$
Adjusted birth weight percentile[a]	42.99 (1.01)
<5th percentile	8.6
5th–10th percentile	6.4
10th–90th percentile	71.0
90th–95th percentile	5.7
95th–100th percentile	8.3
Gestational age	
< 20 completed wk	0.0
20–23 completed wk	0.3
24–27 completed wk	0.4
28–31 completed wk	0.6
32–36 completed wk	7.2
≥ 37 completed wk	91.6
Maternal age	
< 20	9.9
20–34	76.2
35–39	11.4
40+	2.5
Paternal age	
< 20	5.3
20–34	70.0
35–39	17.3
40+	7.3
Maternal race/ethnicity	
Non-Hispanic white	46.0
Non-Hispanic black	10.2
Hispanic	36.9
Other	6.9
Paternal race/ethnicity	
Non-Hispanic white	43.1
Non-Hispanic black	10.9
Hispanic	38.0
Other	8.0
Maternal education	
0–11 (none/primary/some secondary)	17.7
12 (completed secondary)	27.1
13+ (college)	55.2
Marital status	
Not married or cohabitating	14.8
Cohabitating	22.2
Married	63.1

Table 1 (Continued)

Characteristics	Mean (SE) or %$_w$
	$N = 1{,}073$
	$N_w = 882$
Maternal BMI	
< 18.5	3.0
18.5–24.9	51.2
25–29.9	22.7
30–34.9	12.5
≥ 35	10.6
Insurance	
No insurance	4.2
Any public/private insurance	47.8
VA/commercial health insurance/HMO	48.1
Maternal smoking status[b]	
Did not smoke	87.2
< 10	6.3
≥ 10	6.5
Alcohol use[c]	
Did not drink	58.8
Drank, no binging	22.9
Binged	18.3
Illicit drug use[d]	
Never used drugs	72.2
Ever used drugs, no addiction	25.7
Ever used drugs, addiction	2.1

Abbreviations: %$_w$, percent of weighted population; BMI, body mass index; HMO, health maintenance organizations; N$_w$, weighted sample size; SE, standard error; VA, Veterans Affairs.

Note: Study population includes singleton, nonanomalous live births ≤42 weeks' gestation with successful testing for at least one maternal or fetal thrombophilia marker.

[a]Birth weight for gestational age percentiles determined from Hadlock's norms.

[b]Average number of cigarettes during 3 months prior to pregnancy.

[c]Alcohol consumption during 3 months prior to pregnancy.

[d]Lifetime drug use.

example, retroplacental hematoma was observed in 4.4% of pregnancies, and perivillous/intervillous fibrinoid deposition occurred in 1.5% of pregnancies. Intraparenchymal thrombus was significantly associated with a higher mean birth weight percentile (present: 51.47, absent: 41.55). Conversely, perivillous/intervillous fibrin/fibrinoid deposition was significantly associated with a lower mean birth weight percentile (present: 26.67, absent: 42.84). The prevalence of markers of fetal vascular malperfusion (fetal vascular thrombi in the chorionic plate and avascular villi) were observed in approximately 7% of examined placentas. Edema was present in less than 1% of examined placentas. None of

Table 2 Birth weight for gestational age percentile by presence of thrombophilia marker

Thrombophilia marker (common homozygous vs. other)	N_w or $\%_w$	Percentile Mean (SE)	p-Value[a]
FV Leiden G508A (rs6025)			
Maternal marker	638		
GG	97.2	43.20 (1.22)	0.14
Other	2.8	33.74 (6.32)	
Fetal marker	668		
GG	97.4	42.72 (1.14)	0.35
Other	2.6	49.20 (6.78)	
FII prothrombin G20210 (rs1799963)			
Maternal marker	644		
GG	98.1	42.63 (1.21)	0.81
Other	1.9	44.83 (8.86)	
Fetal marker	668		
GG	98.5	43.03 (1.13)	0.11
Other	1.5	56.40 (8.40)	
PAI-1 4G/5G in/del (rs1799768)			
Maternal marker	592		
5G/5G	37.8	38.85 (1.81)	0.03
Other	62.2	44.36 (1.65)	
Fetal marker	661		
5G/5G	38.7	41.78 (1.75)	0.41
Other	61.3	43.66 (1.47)	
MTHFR A1298C (rs1801131)			
Maternal marker	610		
AA	61.5	42.20 (1.55)	0.79
Other	38.5	42.85 (1.94)	
Fetal marker	711		
AA	59.7	42.70 (1.42)	0.74
Other	40.3	43.42 (1.69)	
MTHFR C677T (rs1801133)			
Maternal marker	597		
CC	44.5	43.03 (1.83)	0.74
Other	55.5	42.19 (1.67)	
Fetal marker	696		
CC	45.8	42.87 (1.60)	0.91
Other	54.2	43.13 (1.52)	
Summary marker variable			
Maternal marker	685		
0 markers	17.4	43.55 (2.74)	0.79
≥ 1 markers	82.6	42.76 (1.27)	
Fetal marker	749		
0 markers	15.4	40.96 (2.55)	0.41
≥ 1 markers	84.6	43.28 (1.16)	

Abbreviations: N_w, weighted sample size; $\%_w$, percent of weighted population; SE, standard error.
Note: Thrombophilia markers (common homozygous vs. other) evaluated in maternal blood and placenta/cord blood in singleton, nonanomalous live births ≤42 weeks' gestation, excluding those without a placental examination.
[a]p-Values calculated using t-test for difference in means.

Table 3 Birth weight for gestational age percentile by presence placental characteristics related to vascular malperfusion

Placental characteristic	N_w or $\%_w$	Percentile Mean (SE)	*p*-Value[a]
Maternal vascular malperfusion			
Retroplacental hematoma	878		
Present	4.4	47.61 (4.23)	0.27
Absent	95.6	42.82 (1.04)	
Parenchymal infarction	879		
Present	16.2	39.66 (2.17)	0.12
Absent	83.8	43.51 (1.13)	
Intraparenchymal thrombus	875		
Present	13.6	51.47 (2.61)	0.00
Absent	86.4	41.55 (1.08)	
Perivillous/intervillous fibrin/fibrinoid deposition	846		
Present	1.5	26.67 (5.88)	0.01
Absent	98.5	42.84 (1.04)	
Fetal vascular malperfusion			
Fetal vascular thrombi in the chorionic plate	878		
Present	7.5	41.36 (3.77)	0.68
Absent	92.5	42.98 (1.05)	
Avascular villi	878		
Present	7.3	42.02 (3.55)	0.80
Absent	92.7	42.94 (1.05)	
Edema (placental hydrops)	879		
Present	0.9	31.22 (8.62)	0.17
Absent	99.1	43.02 (1.02)	

Abbreviations: N_w, weighted sample size; $\%_w$, percent of weighted population; SE, standard error.

Note: Restricted to singleton, nonanomalous live births ≤42 weeks' gestation, excluding those with no successful thrombophilia testing.

[a]*p*-Values calculated using *t*-test for difference in means.

the placental characteristics related to fetal vascular malperfusion were associated with a statistically significant difference in mean birth weight percentile.

Thrombophilia Markers and Placental Characteristics
Although not statistically significant, the prevalence of an intraparenchymal thrombus was at least twice as common in placentas of women who had the FV Leiden mutation (present: 23.6%, absent: 13.2%) and in the placentas of infants who also had the FV Leiden mutation (present: 30.4%, absent: 13.3%; ►**Table 4**). Similarly, avascular villi were more than twice as common if the mother or infant had the FV Leiden mutation. In contrast, perivillous/intervillous fibrin was significantly less common in pregnancies in which the mother or the infant had the FV Leiden mutation (maternal present: 0.2%, absent: 1.8%; fetal present: 0.0%, absent: 1.5%). Furthermore, fetal vascular thrombi in the chorionic plate and edema were significantly less frequent if the mother carried the FV Leiden mutation. FII prothrombin in mothers was associated with lower prevalence of parenchymal infarction, perivillous/intervillous fibrin, and fetal vascular

thrombi in the chorionic plate, but a nonsignificant increase in the prevalence of edema. Other thrombophilia polymorphisms were generally not associated with differences in the prevalence of placental characteristics with the exception that fetal vascular thrombi were less common in mothers with PAI-1, perivillous/intervillous fibrin/fibrinoid deposition was more common in mothers with the MTHFR A1298C mutation, and parenchymal infarction was more common in mothers with the MTHFR C677T mutation.

Analysis Results: Maternal Models
When inherited thrombophilia was categorized as zero mutations present versus one or more mutations present (Models 1 and 3), there was no association between thrombophilia and adjusted birth weight percentile (►**Table 5**). This was consistent regardless of whether or not the placental characteristics of interest were included in the model. In the models that included the placental characteristics (Models 1 and 2), parenchymal infarction and perivillous/intervillous fibrin/fibrinoid deposition were associated with a decrease in adjusted birth weight percentile, while intraparenchymal thrombus

Table 4 Presence of placental characteristics related to vascular malperfusion by presence of thrombophilia markers of interest

The table reports percent of weighted population (%w) for each placental characteristic, stratified by presence/absence of each thrombophilia marker (maternal and fetal markers), with p-Values.

Placental characteristic	FVL Mat Present %w	FVL Mat Absent %w	FVL Mat p	FVL Fet Present %w	FVL Fet Absent %w	FVL Fet p	FII Mat Present %w	FII Mat Absent %w	FII Mat p	FII Fet Present %w	FII Fet Absent %w	FII Fet p	PAI-1 Mat Present %w	PAI-1 Mat Absent %w	PAI-1 Mat p	PAI-1 Fet Present %w	PAI-1 Fet Absent %w	PAI-1 Fet p	A1298C Mat Present %w	A1298C Mat Absent %w	A1298C Mat p	A1298C Fet Present %w	A1298C Fet Absent %w	A1298C Fet p	C677T Mat Present %w	C677T Mat Absent %w	C677T Mat p	C677T Fet Present %w	C677T Fet Absent %w	C677T Fet p
(N / Nw)	22/18	765/621		18/17	787/651		16/12	776/632		13/10	786/658		443/368	288/224		308/256	488/405		288/235	463/375		342/286	504/425		392/331	339/266		432/378	396/319	
Maternal vascular malperfusion																														
Retroplacental Hematoma	7.2	4.4	0.57	6.0	5.2	0.89	5.1	4.2	0.85	6.2	5.0	0.85	3.8	4.6	0.65	4.5	5.1	0.74	5.7	3.7	0.23	4.5	5.1	0.70	4.5	4.4	0.95	4.8	4.6	0.94
Parenchymal Infarction[c]	23.4	16.8	0.49	34.4	16.6	0.18	0.0	16.9	0.00	22.2	16.8	0.70	17.6	15.8	0.57	19.7	14.1	0.06	18.1	16.5	0.61	17.6	15.9	0.54	20.1	12.9	0.02	18.8	15.5	0.24
Intraparenchymal Thrombus	23.6	13.2	0.29	30.4	13.3	0.13	7.8	13.6	0.45	32.1	14.0	0.27	10.7	14.8	0.14	13.2	15.4	0.42	13.8	13.5	0.92	13.0	14.8	0.47	13.0	14.5	0.60	13.3	15.2	0.46
Perivillous/intervillous fibrin/fibrinoid deposition	0.2	1.8	0.03	0.0	1.5	0.02	0.0	1.8	0.01	0.0	1.4	0.03	1.6	2.7	0.40	1.7	1.0	0.46	3.7	0.9	0.03	0.8	1.4	0.45	1.5	2.2	0.55	0.9	2.0	0.24
Fetal vascular malperfusion																														
Fetal vascular thrombi in the chorionic plate	0.0	7.3	0.00	7.6	8.4	0.92	1.1	7.3	0.01	0.0	8.8	0.00	5.2	10.9	0.03	7.2	10.0	0.24	7.8	6.9	0.68	7.7	8.3	0.77	7.3	7.4	0.96	8.6	7.7	0.70
Avascular villi[d]	13.7	7.4	0.41	19.3	7.1	0.19	7.8	7.5	0.97	13.1	7.3	0.64	7.2	8.9	0.46	9.0	6.4	0.19	9.6	6.9	0.23	9.3	7.0	0.25	8.3	7.4	0.67	8.1	7.1	0.61
Edema (placental hydrops)	0.0	1.1	0.02	0.0	0.5	0.08	10.5	1.0	0.36	0.0	0.3	0.07	1.3	1.2	0.94	0.7	0.3	0.48	1.3	1.2	0.83	0.0	0.9	0.06	0.9	1.4	0.52	0.6	0.5	0.85

Abbreviations: Nw, weighted sample size; %w, percent of weighted population.

Note: Thrombophilia markers (common homozygous vs. other) evaluated in maternal blood and placenta/cord blood in singleton, nonanomalous live births ≤42 weeks' gestation.

[a]Common homozygous versus other.

[b]p-Values calculated using chi-square test for difference in proportions.

[c]Any parenchymal infarction (focal, multifocal, diffuse) versus none.

[d]Any avascular villi (focal, multifocal, diffuse) versus none.

Table 5 Regression coefficients for birth weight for gestational age percentile modeled as a function of presence of maternal thrombophilia markers and placental characteristics related to maternal vascular malperfusion

All births	Thrombophilia		Retroplacental hematoma		Parenchymal infarction[a]		Intraparenchymal thrombus		Perivillous/intervillous fibrin/fibrinoid deposition	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Model 1[b,c]	−0.26	−6.20, 5.67	7.57	−1.81, 16.95	−6.72	−12.02, −1.43	10.76	4.10, 17.43	−16.25	−28.74, −3.75
Model 2[d]	–		3.55	−5.44, 12.53	−4.99	−9.85, −0.13	11.39	5.59, 17.20	−16.32	−27.56, −5.08
Model 3[b,e]	−1.61	−7.60, 4.37	–	–	–	–	–	–	–	–
Model 4[f]			12.55	1.86, 23.24	−4.70	−10.95, 1.55	12.67	4.68, 20.65	−17.92	−29.86, −5.99
FV Leiden	−13.16	−25.50, −0.82								
FII prothrombin	−0.09	−17.82, 17.64								
PAI-1	4.87	−0.62, 10.35								
MTHFR A1298C	0.70	−4.88, 6.29								
MTHFR C677T	−1.73	−7.25, 3.79								
Model 5[g]			–	–	–	–	–	–	–	–
FV Leiden	−13.08	−24.75, −1.40								
FII Prothrombin	3.07	−13.74, 19.87								
PAI-1	5.18	−0.27, 10.62								
MTHFR A1298C	−0.06	−5.51, 5.39								
MTHFR C677T	−1.19	−6.66, 4.28								

Abbreviations: CI, confidence interval; β, regression coefficient.

Note: Thrombophilia markers (common homozygous vs. other) evaluated in maternal blood in singleton, nonanomalous live births ≤42 weeks' gestation.

[a]Any parenchymal infarction (focal, multifocal, diffuse).

[b]Thrombophilia modeled as presence of 0 versus 1 or more markers in maternal blood.

[c]Model contains thrombophilia marker, placental variables, and covariates (maternal age, maternal race/ethnicity).

[d]Model contains placental variables and covariates (maternal age, maternal race/ethnicity).

[e]Model contains thrombophilia marker and covariates (maternal age, maternal race/ethnicity).

[f]Model contains individual thrombophilia markers, placental variables, and covariates (maternal age, maternal race/ethnicity).

[g]Model contains individual thrombophilia markers and covariates (maternal age, maternal race/ethnicity).

was associated with an increase in adjusted birth weight percentile. There was no association between retroplacental hematoma and adjusted birth weight percentile. These results were consistent regardless of whether or not thrombophilia was included in the model.

When each maternal thrombophilia mutation was included separately in the model (Models 4 and 5), FV Leiden was associated with a 13-point decrease in adjusted birth weight percentile regardless of whether or not placental characteristics were included in the model. There was no statistically significant association between any other inherited thrombophilia and adjusted birth weight percentile.

Analysis Results: Fetal Models

There was no statistically significant association between the summary thrombophilia variable or the individual thrombophilia mutations and adjusted birth weight percentile (►Table 6). These results were consistent in the models with and without adjustment for placental characteristics related to fetal vascular malperfusion. Similarly, none of the placental characteristics included in these models were

significantly associated with adjusted birth weight percentile, regardless of adjustment for thrombophilia variables.

Analysis Results: Small for Gestational Age

When logistic regression was used to model the dichotomous SGA outcome, there were no statistically significant associations between the summary thrombophilia variable and the individual thrombophilia variables with SGA in the maternal or fetal models, regardless of whether or not placental characteristics were included in the model (►Tables 7 and 8). In the fully adjusted maternal model, the odds ratio (OR) for the association between SGA and the FV Leiden mutation was 1.43 (95% confidence interval [CI]: 0.33, 6.11; ►Table 7). In the maternal models, intraparenchymal thrombus was consistently significantly associated with a protective effect against SGA (OR, fully adjusted model: 0.35; 95% CI: 0.14, 0.87).

Discussion

Based on our results, we suggest that maternal FV Leiden may be associated with reduced birth weight percentile and

Table 6 Regression coefficients for birth weight for gestational age percentile modeled as a function of presence of fetal thrombophilia markers and placental characteristics related to fetal vascular malperfusion

All births	Thrombophilia		Fetal vascular thrombi in the chorionic plate		Avascular villi[a]		Edema	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Model 1[b,c]	0.56	−5.21, 6.32	−5.47	−13.42, 2.47	−0.55	−8.53, 7.43	−3.63	−32.06, 24.79
Model 2[d]	–	–	−2.83	−10.79, 5.14	−1.52	−9.06, 6.02	−6.89	−25.24, 11.46
Model 3[b,e]	0.58	−5.15, 6.31	–	–	–	–	–	–
Model 4[f]			−5.09	−13.34, 3.16	0.03	−9.25, 9.31	−5.05	−32.94, 22.85
FV Leiden	5.55	−7.69, 18.79						
FII Prothrombin	5.85	−13.39, 25.08						
PAI-1	1.36	−3.73, 6.46						
MTHFR A1298C	−0.72	−5.98, 4.53						
MTHFR C677T	−1.72	−7.14, 3.69						
Model 5[g]			–	–	–	–	–	–
FV Leiden	5.39	−8.18, 18.95						
FII Prothrombin	6.19	−12.83, 25.20						
PAI-1	1.45	−3.64, 6.55						
MTHFR A1298C	−0.36	−5.63, 4.92						
MTHFR C677T	−1.28	−6.69, 4.14						

Abbreviations: CI, confidence interval; β, regression coefficient.
Note: Thrombophilia markers (common homozygous vs. other) evaluated in placenta/cord blood in singleton, nonanomalous live births ≤42 weeks' gestation.
[a]Any avascular villi (focal, multifocal, diffuse).
[b]Thrombophilia modeled as presence of 0 versus 1 or more markers in maternal blood.
[c]Model contains thrombophilia marker, placental variables, and covariates (maternal age, maternal race/ethnicity, paternal age, paternal race/ethnicity).
[d]Model contains placental variables and covariates (maternal age, maternal race/ethnicity, paternal age, paternal race/ethnicity).
[e]Model contains thrombophilia marker and covariates (maternal age, maternal race/ethnicity, paternal age, paternal race/ethnicity).
[f]Model contains individual thrombophilia markers, placental variables, and covariates (maternal age, maternal race/ethnicity, paternal age, paternal race/ethnicity).
[g]Model contains individual thrombophilia markers and covariates (maternal age, maternal race/ethnicity, paternal age, paternal race/ethnicity).

Table 7 Odds ratios for small for gestational age (<10th percentile) as a function of presence of maternal thrombophilia markers and placental characteristics related to maternal vascular malperfusion

All births	Thrombophilia		Retroplacental hematoma		Parenchymal infarction[a]		Intraparenchymal thrombus		Perivillous/ intervillous fibrin/fibrinoid deposition	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Model 1[b,c]	1.18	0.65, 2.14	0.42	0.13, 1.34	1.13	0.64, 1.99	0.27	0.11, 0.65	2.26	0.63, 8.08
Model 2[d]	–	–	0.62	0.21, 1.80	1.16	0.69, 1.94	0.28	0.13, 0.60	2.42	0.67, 8.69
Model 3[b,e]	1.23	0.69, 2.18	–	–	–	–	–	–	–	–
Model 4[f]			0.37	0.09, 1.55	1.21	0.66, 2.22	0.35	0.14, 0.87	2.08	0.47, 9.11
FV Leiden	1.43	0.33, 6.11								
FII Prothrombin	0.82	0.20, 3.30								
PAI-1	1.12	0.66, 1.88								
MTHFR A1298C	0.96	0.56, 1.65								
MTHFR C677T	1.18	0.69, 2.03								
Model 5[g]			–	–	–	–	–	–	–	–
FV Leiden	1.63	0.48, 5.52								
FII Prothrombin	0.73	0.19, 2.76								
PAI-1	1.06	0.64, 1.75								
MTHFR A1298C	1.00	0.60, 1.66								
MTHFR C677T	1.16	0.69, 1.94								

Abbreviations: CI, confidence interval; OR, odds ratio.
Note: Thrombophilia markers (common homozygous vs. other) evaluated in maternal blood in singleton, nonanomalous live births ≤42 weeks' gestation.
[a]Any parenchymal infarction (focal, multifocal, diffuse).
[b]Thrombophilia modeled as presence of 0 versus 1 or more markers in maternal blood.
[c]Model contains thrombophilia marker, placental variables, and covariates (maternal age, maternal race/ethnicity).
[d]Model contains placental variables and covariates (maternal age, maternal race/ethnicity).
[e]Model contains thrombophilia marker and covariates (maternal age, maternal race/ethnicity).
[f]Model contains individual thrombophilia markers, placental variables, and covariates (maternal age, maternal race/ethnicity).
[g]Model contains individual thrombophilia markers and covariates (maternal age, maternal race/ethnicity).

may also be associated with placental changes, including parenchymal infarction, intraparenchymal thrombus, and avascular villi. These placental changes do not appear to account for the observed decrease in fetal growth associated with maternal FV Leiden. Other maternal thrombophilia mutations and all fetal thrombophilia mutations evaluated do not appear to affect birth weight percentile. These results were consistent in a subanalysis restricted to term births (results not shown).

The prevalence of each of the five thrombophilia mutations evaluated is slightly lower than that has been reported in the literature for populations of European descent.[3,18,19] However, this is expected, as the SCRN study was conducted in a diverse population and these mutations are most common in those of European descent.[18] Similarly, the mean adjusted birth weight percentile of our study population was lower than expected, likely due to the use of Hadlock's growth curves, which are based on middle-class white women.[15]

The measure of association for FV Leiden in our SGA analysis was consistent in magnitude with a systematic review and meta-analysis of 32 studies on the association between maternal FV Leiden mutation and SGA (pooled OR: 1.40; 95% CI: 1.18, 1.67).[5] Our lack of statistical significance may be due to small sample size. Of the 22 mothers with the FV Leiden mutation, only five delivered SGA infants. Several studies have reported no association between other thrombophilia mutations (FII prothrombin, MTHFR A1298C, and MTHFR C677T) and fetal growth restriction, which is consistent with our results.[6,20] The consistency of our results with the literature strengthens the likelihood that our observed association with FV Leiden is a real association and not a result of uncontrolled confounding or another potential bias.

One study evaluating placental abnormalities associated with FV Leiden mutation reported that fetal FV Leiden mutation is associated with avascular villi and maternal FV Leiden mutation is associated with increased numbers of syncytial knots and hypervascular villi.[21] Our study also found that avascular villi were more than twice as common in the presence of fetal FV Leiden mutation. To our knowledge, no studies have considered placental function and characteristics when evaluating the relationship between inherited thrombophilia and fetal growth restriction, and

Table 8 Odds ratios for small for gestational age (<10th percentile) as a function of presence of thrombophilia markers in placenta/cord blood and placental characteristics related to fetal vascular malperfusion

All Births	Thrombophilia		Fetal vascular thrombi in the chorionic plate		Avascular villi[a]		Edema	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Model 1[b,c]	1.28	0.73, 2.26	1.90	0.98, 3.72	1.09	0.56, 2.14	1.64	0.19, 14.27
Model 2[d]	–	–	1.43	0.75, 2.74	1.11	0.59, 2.09	0.36	0.07, 1.85
Model 3[b,e]	1.32	0.74, 2.34	–	–	–	–	–	–
Model 4[f]			1.77	0.88, 3.59	1.13	0.54, 2.36	2.58	0.25, 26.99
FV Leiden	0.60	0.13, 2.76						
FII prothrombin	0.41	0.05, 3.54						
PAI-1	0.95	0.58, 1.54						
MTHFR A1298C	1.03	0.61, 1.73						
MTHFR C677T	1.37	0.79, 2.35						
Model 5[g]			–	–	–	–	–	–
FV Leiden	0.61	0.13, 2.84						
FII prothrombin	0.39	0.05, 3.33						
PAI-1	0.94	0.58, 1.52						
MTHFR A1298C	1.03	0.62, 1.74						
MTHFR C677T	1.35	0.79, 2.33						

Abbreviations: CI, confidence interval; OR, odds ratio.
Note: Thrombophilia markers (common homozygous vs. other) evaluated in placenta/cord blood in singleton, nonanomalous live births ≤42 weeks' gestation.
[a]Any avascular villi (focal, multifocal, diffuse).
[b]Thrombophilia modeled as presence of 0 versus 1 or more markers in maternal blood.
[c]Model contains thrombophilia marker, placental variables, and covariates (maternal age, maternal race/ethnicity, paternal age, paternal race/ethnicity).
[d]Model contains placental variables and covariates (maternal age, maternal race/ethnicity, paternal age, paternal race/ethnicity).
[e]Model contains thrombophilia marker and covariates (maternal age, maternal race/ethnicity, paternal age, paternal race/ethnicity).
[f]Model contains individual thrombophilia markers, placental variables, and covariates (maternal age, maternal race/ethnicity, paternal age, paternal race/ethnicity).
[g]Model contains individual thrombophilia markers and covariates (maternal age, maternal race/ethnicity, paternal age, paternal race/ethnicity).

few studies have considered the role of fetal inherited thrombophilia.

One limitation of this study includes relatively low power due to low prevalence of the placental characteristics of interest and low prevalence of some mutations, particularly FV Leiden and FII prothrombin. This is true even though the SCRN population has a relatively large sample size and had data on placental characteristics and genetic markers on nearly 1,000 live births. In an effort to increase sample size, we evaluated the effect of thrombophilia in all live births with thrombophilia testing (not restricted to those with a placental examination) and our results for maternal FV Leiden were not consistent (β: −2.96; 95% CI: −14.50, 8.58). This may also be a result of small sample size. In the total sample, there were 33 births with the maternal FV Leiden mutation and 22 in the sample restricted to those with a completed placental examination. This may be a selection issue, but this seems unlikely given that the proportion of those with maternal FV Leiden was consistent in

both samples (2.8%). Additionally, 93.4% of women consented to a placental examination and in the majority who did not have a complete examination, the placenta had been previously discarded. Owing to the low prevalence, we were unable to evaluate heterozygotes and homozygotes separately. Additionally, as this was a preliminary study, we did not adjust for multiple testing. We also focused our results on magnitude rather than statistical significance, which is consistent with the American Statistical Association's stance on p-values.[17] These results should be evaluated in populations with a higher prevalence of FV Leiden mutation.

Another limitation is the use of birth weight percentile as an indicator of fetal growth. While fetal growth implies longitudinal measures of growth, birth weight at delivery reflects cumulative growth over the course of the pregnancy. Use of birth weight percentile is advantageous over birth weight because it accounts for variation in size by gestational age. A change in birth weight percentile has a different implication for magnitude of change in actual birth weight

at different gestational ages. Although not a perfect measure of fetal growth, the use of continuous birth weight percentile is also a strength. Previous studies have dichotomized fetal growth at various cutoffs (<5th percentile, <10th percentile, <2 standard deviations below the mean) or based on abnormal blood flow from Doppler readings. Variations in the definition used may explain the conflicting results.[5,22] Use of a continuous measure from published norms enhances the comparability and reproducibility of our results.

Additional strengths of this study include standardized protocol for sample collection, storage, and analysis to determine the presence of the thrombophilia markers. Similarly, all study pathologists used a standardized placental examination protocol. Additionally, the SCRN study included a relatively large population that was both geographically and racially diverse.

Maternal FV Leiden appears to negatively affect fetal growth, independent of placental characteristics related to maternal vascular malperfusion. Further research is needed on populations with a higher prevalence of FV Leiden to evaluate this association with improved precision.

Conflict of Interest
None declared.

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